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Demonstration of Bioaugmentation at Kelly AFB, TX

Final Report
Cost & Performance Report

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13. ABSTRACT *(Maximum 200 words)* Bioaugmentation is an innovative technology designed to promote complete reductive dechlorination at sites where natural attenuation or enhanced anaerobic dechlorination cannot achieve the level of contaminant destruction to protect human health and the environment. Enhanced anaerobic bioremediation through the addition of electron donors and/or other nutrients is being demonstrated in the field at an increasing number of sites where the geochemical environment cannot support natural attenuation. While the technology has been able to achieve the anaerobic conditions necessary for halo respiration, the sites do not exhibit complete dechlorination, most commonly due to microbial limitations. Recently, bioaugmentation has been demonstrated successful for achieving complete dechlorination at sites where delivery of donor/nutrient amendments resulted in limited success. One such site is the Building 360 Site at Kelly AFB, Texas where dechlorination of trichloroethylene (TCE) was demonstrated to hold up at *cis*-dichloroethylene. The Environmental Security Technology Certification Program funded a study to test the robustness of the bioaugmentation technology. The objectives were to investigate the survivability of the KB1 culture, evaluate any residual dechlorinating activity, attempt to reestablish the level of activity to pre-shutdown levels, and to stress the culture by adding sulfate. Gene probe analysis on groundwater samples collected across the augmented test plot all tested positive for the presence KB1, none of the samples from the non-augmented control plot tested positive. Complete TCE dechlorination was observed in the vicinity of the test plot suggesting that the KB1 culture was utilizing a source of electron donor already in the groundwater although the level of dechlorination appeared to be lower than the level during donor addition. The test system was put back online under the same operating conditions used during the RTDF demonstration and the level of reductive dechlorination quickly rebounded. Sulfate was added to establish an initial in-situ concentration of 400 mg/L. Significant amount of the sulfate was reduced, decreasing the concentration to 50 mg/L within 6 weeks. No apparent impact on the dechlorination activity was observed from the added sulfate. The implications from these data are that 1) the KB1 culture was very robust being able to compete with, and survive among, the indigenous microbial population, and 2) bioaugmentation may not require continuous attention following inoculation at sites where the natural attenuation requirements are met.

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Acronyms and Abbreviations

AFB	Air Force Base
bgs	below ground surface
DCE	dichloroethene
Dhc	*Dehalococcoides ethenogenes*
DO	dissolved oxygen
DoD	(United State) Department of Defense
ECD	electron capture detector
ESTCP	Environmental Security Technology Certification Program
FID	flame ionization detector
GC	gas chromatograph
gpm	gallons per minute
HCl	hydrochloric acid
lf	linear feet
MCL	maximum contaminant level
NAAS	Naval Air Auxiliary Station
NAPL	nonaqueous-phase liquid
NAS	Naval Air Station
O&M	operations and maintenance
ORNL	Oak Ridge National Laboratory
ORP	oxidation-reduction potential
PCE	tetrachloroethene
ppb	parts per billion
PPE	personal protective equipment
PVC	polyvinyl chloride
RNA	ribonucleic acid
RTDF	Remediation Technologies Development Forum
SOP	Standard Operating Procedure
TCE	trichloroethene

USDA	United States Department of Agriculture
U.S. EPA	United States Environmental Protection Agency
VC	vinyl chloride
VFA	volatile fatty acid
VOC	volatile organic compound

Executive Summary

Chlorinated ethenes such as tetrachloroethene (PCE) and trichloroethene (TCE) are some of the most common groundwater contaminants found at Department of Defense (DoD) facilities. In addition to their common presence, these compounds are persistent under most natural geochemical conditions at these contaminated sites. Remediation of these sites through biodegradation of the chlorinated ethenes is a promising alternative at many of the sites. Reductive dechlorination is the primary pathway for biodegradation of chlorinated solvents. With this pathway, the chlorine atoms on the ethenes are sequentially replaced by hydrogen atoms through a biologically-mediated process. Generally, the hydrogen is generated through fermentation of an electron donor. Although many microorganisms are capable of mediating the reductive dechlorination process, only *Dehalococcoides ethenogenes* is known to completely reduce PCE and TCE to ethene. Unfortunately, *D. ethenogenes* is not present at all choroethene-contaminated sites and the reductive dechlorination process stalls at cis-1,2-dichloroethene (c-DCE). Under conditions such as these, the application of enriched cultures (such as KB-1 and Pinellas) containing *D. ethenogenes* or closely related microorganisms is used to complete the reductive dechlorination process.

The primary objective of the demonstration was to determine if complete reductive dechlorination could be stimulated through the introduction of a culture (KB-1) known to contain halorespiring bacteria (*D. ethenogenes*). Secondary objectives involved testing the robustness of the applied culture by depriving it of electron donor and adding sulfate to the system. Samples were collected and analyses were performed at a frequency to evaluate the objectives of the demonstration. The results of the chemical analyses indicated that the complete dechlorination was achieved through the addition of the microbial culture. Each of the performance objectives was met during the demonstration at Kelly Air Force Base (AFB). The data indicate that the KB-1 culture was capable of stimulating complete reductive dechlorination. In addition, it was determined that the KB-1 culture, specifically *D. ethenogenes,* was fairly robust with the elimination of the electron donor and the addition of the sulfate from/to the system.

In 1976, the United States Environmental Protection Agency (U.S. EPA) designated PCE and TCE as priority pollutants. The Safe Drinking Water Act Amendments of 1986 strictly regulate both of these compounds; each has a maximum contaminant level (MCL) in drinking water of 5 parts per billion (ppb) (U.S. EPA, 1996). When concentrations of these compounds at a contaminated site are too high, remedial action is required to lower the concentration and reduce the risk to human health and the environment.

Bioaugmentation was successfully demonstrated for achieving complete dechlorination at Kelly AFB where delivery of donor/nutrient amendments resulted in limited success. At Kelly AFB, dechlorination of PCE was demonstrated to hold up at *c*-DCE with only the addition of an electron donor. After the aquifer was augmented with KB-1, a prepared culture of halorespiring bacteria, complete dechlorination of PCE to ethene was observed.

Following the successful demonstration of the bioaugmentation technology, the robustness of the KB-1 culture was tested through the deprivation of electron donor and then the addition of

sulfate. The objectives were to investigate the survivability of the KB-1 culture, evaluate any residual dechlorinating activity, attempt to reestablish the level of activity to pre-shutdown levels, and to stress the culture by adding sulfate. After approximately one year without the addition of the electron donor, gene probe analysis on groundwater samples collected across the augmented test plot all tested positive for the presence *D. ethenogenes*, and none of the samples from the non-augmented control plot tested positive. Complete PCE dechlorination was observed in one well inside the test plot suggesting that the *D. ethenogenes* was utilizing a source of electron donor already in the groundwater. After the addition of the electron donor, complete reductive dechlorination was quickly observed in all of the wells.

Sulfate was added to establish an initial in-situ concentration of 600 mg/L. A significant amount of the sulfate was reduced, decreasing the concentration to 50 to 60 mg/L within 6 weeks. No apparent impact on the dechlorination activity was observed from the added sulfate.

The implications from these data are that (1) the KB-1 culture was very robust being able to compete with, and survive among, the indigenous microbial population, and (2) bioaugmentation may not require continuous attention following inoculation at sites where the natural attenuation requirements are met.

1. Introduction

1.1 Background

Chlorinated ethenes such as tetrachloroethene (PCE) and trichloroethene (TCE) are some of the most common groundwater contaminants found at United States Department of Defense (DoD) facilities. In addition, these compounds are persistent under most natural geochemical conditions. Remediation of these contaminants through biodegradation after appropriate geochemical conditions have been achieved is a promising alternative at many of DoD sites.

Reductive dechlorination is the primary pathway for biodegradation of chlorinated solvents. Through this pathway, the chlorine atoms on the ethenes are sequentially replaced by hydrogen atoms through a biologically-mediated process. Generally, the hydrogen is generated through fermentation of an electron donor. Although many microorganisms are capable of mediating the reductive dechlorination process, only *Dehalococcoides ethenogenes* is known to completely reduce PCE and TCE to ethene. Unfortunately, at sites that lack the presence of this microorganism, the reductive dechlorination frequently process stalls at *cis*-1,2-dichloroethene (DCE). Therefore, the application of enriched cultures containing *D. ethenogenes* or closely related microorganisms may be used to complete the reductive dechlorination process.

1.2 Objectives

The primary objective of this project was to demonstrate the application of a culture known to dechlorinate chloroethenes in order to determine if complete dechlorination to ethene could be achieved. To achieve this objective, microcosm studies were conducted by GE using soils collected from a chloroethene-contaminated site at Naval Air Station (NAS) Fallon, NV and the Pinellas culture. Due to the extremely high sulfate concentrations in the groundwater from the site, complete dechlorination could not be achieved, and another site had to be selected to demonstrate the technology. The Building 360 site at Kelly Air Force Base (AFB), TX, was selected as the secondary site. Although the successful application of bioaugmentation had already been demonstrated at this site, it was selected to determine if reductive dechlorination could be continued after a period during which electron-donating substrate was not added to the system.

1.3 Regulatory Drivers

In 1976, the United States Environmental Protection Agency (U.S. EPA) designated PCE and TCE as priority pollutants. The Safe Drinking Water Act Amendments of 1986 strictly regulate both of these compounds; each has a maximum contaminant level (MCL) in drinking water of 5 parts per billion (ppb) (U.S. EPA, 1996). When concentrations of these compounds at a contaminated site are too high, remedial action is required to lower the concentration and reduce the risk to human health and the environment.

1.4 Stakeholder/End-User Issues

Design and application of bioaugmentation technology consists of installing/using simple components that are readily available (as with the various other biostimulation technologies). This technology however requires the introduction of organisms specifically selected/grown to operate in subsurface environments where native organisms either are absent or are not robust enough to be simply biostimulated. As such bioaugmentation techniques need not be applied

when capable, native degradative microorganisms are present in the subsurface to be treated. Microcosm tests can determine if this is the case and these tests are best left to expert bioremediation practitioners/companies. Initial design and installation of a bioaugmentation system would require some specialized knowledge and it is the express purpose of the Remediation Technologies Development Forum (RTDF), Bioremediation Consortium (a partner in this technology demonstration) to educate the public with respect to the knowledge needed to appropriately choose such a technology. They can be reached at: <http://www.rtdf.org/public/biorem/biodocsp.htm> . Several documents have been placed there to assist remedial program managers. Once the technology has been set in place, most properly trained field technicians could continue to operate the system in the field in most instances.

2. Technology Description

2.1 Technology Development and Application

Chlorinated ethenes are widely used as solvents, cleaners, and degreasing agents. As a result of spills and past disposal practices, these compounds are often found as contaminants in groundwater, soil, and sediments. Standard remedial approaches have proven to be ineffectual and costly at removing these substances from the environment. Within the last 15 years, basic research on natural microbial dechlorination mechanisms has suggested that the destruction of chlorinated compounds may be practically achieved at some sites by stimulating bacterial reductive dechlorination in the field.

Stimulation of microbial reductive dechlorination is achieved through the injection of electron-donating substrates and nutrients into the groundwater to produce proper oxidation/reduction conditions. Although stimulated biodegradation of chloroethenes may be an effective method of site remediation at many sites, there are instances where complete degradation of PCE and TCE to ethene is not possible through the addition of electron donors alone. In these cases, the degradation of PCE and TCE often stops at *cis*-DCE or vinyl chloride, resulting in the accumulation of these degradation components. The partial dechlorination of PCE and TCE may be caused by the absence of dechlorinating microorganisms (i.e., dehalorespiring microorganisms).

The microorganism *Dehalococcoides ethenogenes* has been shown to completely reduce PCE and DCE to ethene, and cultures that contain phylogenetically-related organisms to *D. ethenogenes* have been produced for application in the field. Examples of such cultures include the Bachman culture, the Pinellas culture and the KB-1 culture. A field demonstration of the Pinellas culture was conducted at Dover AFB, and indicated that the dechlorination of *cis*-DCE to ethene occurred only after the addition of the culture.

2.2 Previous Testing of Technology

Demonstration of the bioaugmentation technology for the in situ treatment of chlorinated ethenes has been conducted at several sites, from bench-scale to field-scale application. Results of these demonstrations and test range from failure to success. Often with the successful demonstrations, the results do not conclusively prove that the complete reductive dechlorination is a direct result of the addition of the culture. The White Paper produced for this project (Development of Bioaugmentation for Groundwater Remediation) presents the state of the technology along with case studies of the demonstrations that have been performed. The White Paper is included as Appendix C of this report.

2.3 Factors Affecting Cost and Performance

Site conditions such as aquifer geochemistry and hydrology are the greatest factors affecting the cost and performance of the technology. For example, the presence of sulfate in the groundwater at NAS Fallon was believed to prevent the reductive dechlorination of the solvents with the indigenous and the applied cultures. The added electron donor was used primarily in the reduction of sulfate, and reductive dechlorination could not proceed. In addition, the high salinity at the NAS Fallon site likely limited the survival and success of the applied culture. The

introduction of a culture into an extreme environment, such as that found at NAS Fallon, likely limits the successful performance of the technology. Other factors such as the effective distribution of the culture across the remediation site, overall survival of the applied culture, and the activity of the culture have an effect on the success of the remediation process through the implementation of bioaugmentation.

2.3.1 Transport and Distribution of Culture. Factors such as hydrogeology and microbial transport affect the distribution of the injected culture and, therefore, the performance of the technology. Major parameters that affect the distance across which bacteria cells are transported include bacterial cell surface properties (surface charge, hydrophobicity, physical structure), cell size, soil characteristics (macropore structure, grain size, organic content, clay type), groundwater chemistry and flowrates, and additives. Generally, microbial transport is inhibited by small grain size distribution of the soil, high organic content, minimal macropore structures and strong positive charge to the soil particles. In addition, Jennings et al. (1995) provide data that suggest microbial transport through the aquifer is related to (1) injection pressure of the culture, (2) the influent substrate loading rates (e.g., the lower the substrate-loading rate the lower the transport of the culture), and (3) nutrient injection. It appears from these studies that continuous injection of nutrients caused the biomass to accumulate or develop near the nutrient injection points.

2.3.2 Survival of the Culture. Survival studies indicate that factors such as pH affect the survivability of cultures (Dybas et al., 1995). Dybas et al. (1995) suggests that pH modification of the aquifer can produce a niche for the introduced culture. For the *Pseudomonas stutzeri* KC that they were investigating, the production of moderately alkaline conditions (pH 7.9 to 8.2) was effective at creating such a niche and increasing the competitiveness of strain KC. The change in pH likely affected concentrations or speciation of metals (that inhibit growth of the cultured microorganisms) in the aquifer. In addition to the speciation and concentrations of metals, other geochemical conditions and predation or competition by indigenous microbes may limit the survivability of the culture.

2.3.3 Culture Activity. Witt et al. (1995) performed a laboratory study to assess the zone of biotransformation downstream from the injection location. The study resulted in the production of a numerical model that can predict the contaminant degradation by the bacterium (*Pseudomonas stutzeri* KC) that was used in the study. The output from the model then was compared to the results of a column study. Both the predicted and experimental results indicated a relatively small zone (50 cm) of contaminant degradation downstream from the location of the microbial injection.

2.3.4 Intimate Contact. As with any in situ aquifer restoration technology, direct contact between the contaminant and the remedial reagent(s) is critical for success. For bioaugmentation to be effective, the added microorganisms must be brought into direct contact with the contaminant. The limitations of microbial transport suggest that it may be desirable to inject the culture and bring the contaminant to the cells. This would favor in situ biological barrier or biofilter configurations, which are set up by injecting the culture into a designated volume of the aquifer to establish an active zone across which the contaminant is either pumped

or allowed to pass through with the flow of groundwater. Designing a system that provides intimate contact requires detailed aquifer testing and evaluation.

2.4 Advantages and Limitations of the Technology

The advantages of bioaugmentation over traditional technologies for chlorinated solvent remediation, such as biostimulation or pump-and-treat, are cost and duration of cleanup project. Bioaugmentation is more cost effective than pump-and-treat technologies due to the high capital and operational costs of pump-and-treat systems. In a comparison of bioaugmentation with biostimulation, bioaugmentation appears favorable because the duration of the remediation project may be shortened when bioaugmentation is used – even if biostimulation is capable of achieving complete dechlorination. Application of a culture to the contaminated aquifer likely will increase the biodegradation rates relative to straight biostimulation. However, simple biostimulation may not achieve the remedial goals of complete reductive dechlorination to ethene.

The main advantages of bioaugmentation for remediation of chlorinated solvents include the following:

1. Bioaugmentation results in contaminant destruction, not simply phase transfer;
2. The technology utilizes the aquifer volume as an in situ bioreactor;
3. In situ destruction of the contaminant may relieve regulatory requirements associated with pumping followed by aboveground treatment;
4. In situ treatment minimizes water disposal and preserves water balance.
5. Bioaugmentation may produce cost savings over traditional technologies i.e., biostimulation and pump-and-treat;
6. Bioaugmentation may produce more favorable results compared to biostimulation.

The main limitations of the bioaugmentation technology include the following:

1. The culture must establish a niche in the aquifer and be able to compete with the indigenous microorganisms for essential nutrients;
2. The application is limited to sites of sufficient permeability to allow manipulation of groundwater flow;
3. The overall effectiveness depends on the ability to distribute the culture adequately in the subsurface.

Bioaugmentation is an innovative technology and the status of regulatory acceptance is unknown.

3. Demonstration Design

3.1 Performance Objectives

The performance objectives for the bioaugmentation demonstrations are provided in Table 3-1. The objectives were classified as Qualitative and Quantitative, and each objective had specific parameters used to measure the success of the objective.

Table 3-1 Performance Objectives for the Bioaugmentation Demonstration

Type of Performance Objective	Primary Performance Criteria	Expected Performance	Actual Performance
Qualitative	Achieve complete dechlorination at NAS Fallon via bioaugmentation	Success	Failure
	Determine if dechlorination would occur after a one-year shutdown period at Kelly AFB	Success	Success
	Determine effects of introduced sulfate on the reductive dechlorination process at Kelly AFB	Evaluate if increasing sulfate concentration inhibit the dechlorination process	Inconclusive
Quantitative	Determine PCE/TCE degradation rates at Kelly AFB	Determine the rate of conversion from PCE/TCE to ethene	Inconclusive

The original objective (when the project was being conducted at NAS Fallon) of this study was to achieve complete dechlorination of PCE at a study site through the introduction of a known dechlorinating culture. NAS Fallon was selected as the demonstration site because repeated attempts at biostimulation had not been successful at the site. The attempt at using bioaugmentation to achieve complete reductive dechlorination was unsuccessful at NAS Fallon; therefore, the study was moved to Kelly AFB where a successful bioaugmentation demonstration had been conducted. With the change in the demonstration location, the objectives of the demonstration were changed. Initially, the objective of the demonstration at Kelly AFB was to determine if complete reductive dechlorination could be produced following a one-year shutdown period (during which no electron donor was injected into the system) and no bioaugmentation had occurred since the initiation of the project. After reductive dechlorination was achieved following the shutdown period, the objective was changed to determine if increasing sulfate concentrations in the aquifer would inhibit reductive dechlorination.

3.2 Selecting Sites

NAS Fallon was initially selected because a number of studies had been performed there for evaluating reductive dechlorination through biostimulation. All of the studies were unsuccessful at achieving dechlorination to ethene. In some of these studies, the reductive dechlorination process could not be initiated, and it the lack of appropriate microbial populations was viewed as a possible reason for the lack of complete dechlorination.

After complete dechlorination could not be achieved at NAS Fallon using the Pinellas culture, it was decided that testing should be conducted at Kelly AFB, where bioaugmentation had successfully been demonstrated. At Kelly AFB, the objective was to determine the robustness of the KB-1 culture that was used at the site. At Kelly AFB, depriving the culture of electron donor for more than a year would test the robustness of the culture. If the culture successfully rebounded and dechlorination was started again, the dechlorination process would be perturbed with the addition of sulfate to the test plot.

3.3 Test Site Description

3.3.1 NAS Fallon. NAS Fallon is located in Churchill County, NV, approximately 6 miles southeast of the town of Fallon and 60 miles east of the city of Reno. Fallon was established as a military facility in 1942 under the Western Defense Program, and the base was commissioned as a Naval Air Auxiliary Station (NAAS) in 1944. In 1972, it was upgraded to a Naval Air Station. NAS Fallon serves as an aircraft weapons delivery and tactical air combat training facility.

The Crash Crew Training Area (Site 1) is located in the southern part of NAS Fallon. The site consisted of an unlined, earthen burn pit; approximately 25 ft in diameter by 3 ft deep that was used to conduct fire-training exercises (see Figure 3-1 Map of Site 1). Previously, two aboveground fuel storage tanks (1,000- and 5,000-gallon capacities) were located approximately 180 ft to the west of the burn pit. The tanks were removed in 1994 (ORNL, 1994). From the mid-1950s to April 1988, the pit was used to burn an estimated 1.1 million gallons of flammable liquids, waste products from the old and new fuel farms, napalm, lubricants, and solvents. It is estimated that 99% of the material burned was fuel and lubricants, and between 1982 and April 1988, only JP-5 jet fuel was burned in the pit (ORNL, 1994).

The Fallon area is in the northwestern part of the Great Basin. Geologic deposits in the area consist of lacustrine sediments interwedged with alluvial and eolian material deposited during the interpluvial periods. Soils at Site 1 consist of fine sand and clay loam to a depth of approximately 6 ft. Underlying those soils are alternating layers of clay, silty/clayey sand, and sand. Groundwater on the base and at Site 1 generally is encountered at 5 to 10 ft below ground surface (bgs). Groundwater at Site 1 contains high dissolved solids and alkalinity, rendering it poor for municipal and agricultural uses.

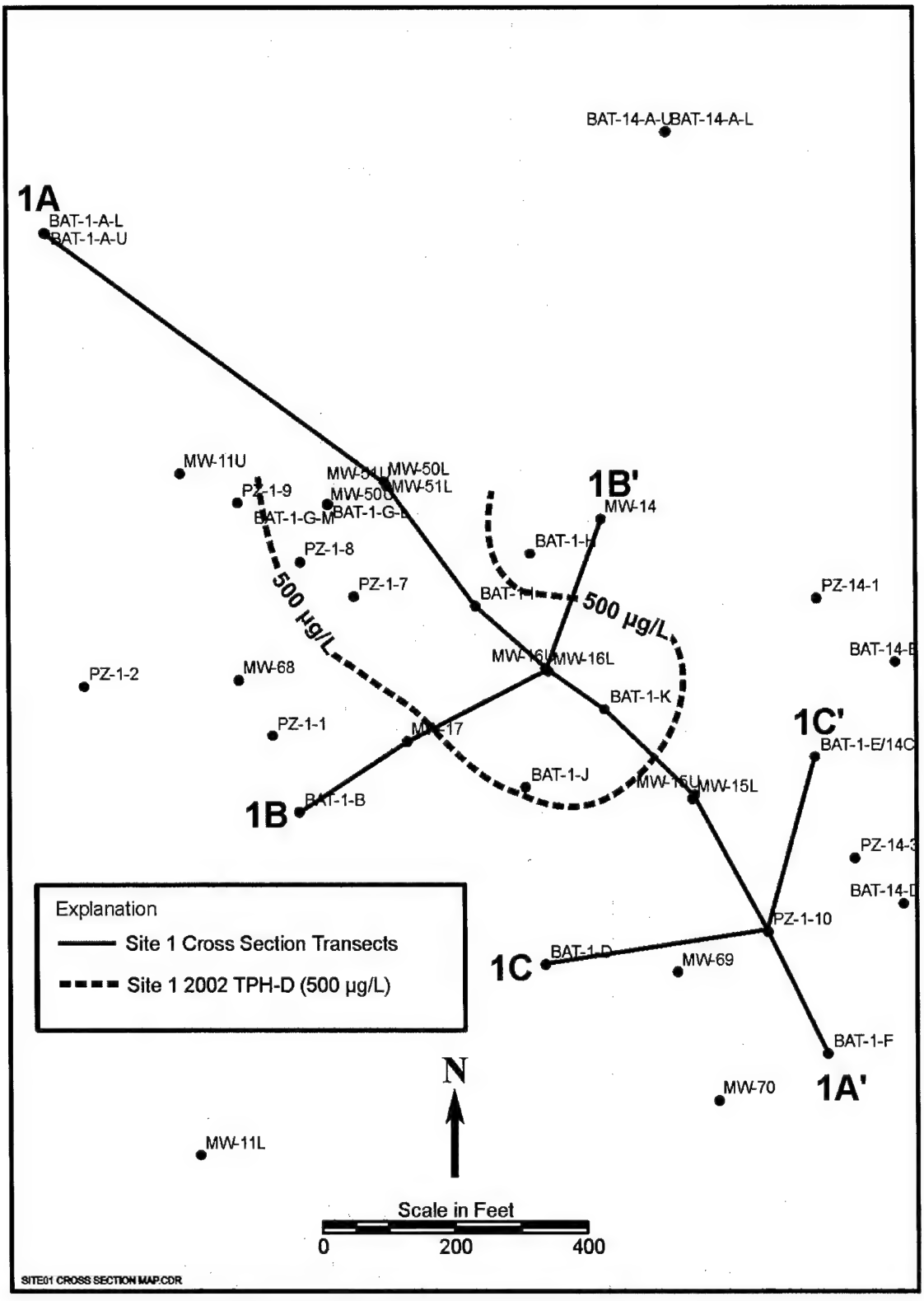

Figure 3-1. Site 1, NAS Fallon, Nevada

In general, the area of contaminated groundwater at Site 1 originates near a former Fire Training Pit and extends hydraulically downgradient to the southeast. Groundwater contaminants at Site 1 include compounds derived from chlorinated solvents and petroleum fuels. For this project, the chlorinated compounds are the contaminants of interest. Chlorinated hydrocarbons detected at Site 1 include PCE, TCE, *cis*-1,2-DCE, *trans*-1,2-DCE, 1,1-DCE, and vinyl chloride (VC). 1,2-DCA also was detected in one well and is likely derived from fuels disposed at the site.

3.3.2 Kelly AFB. The location for the demonstration is situated in the courtyard of Building 360 (see Figure 3-2 Map of Building 360 Site). The demonstration site was selected for the bioaugmentation study based on the presence and concentrations of the contaminants, access to an existing test infrastructure, site hydrogeology/geology, and site logistics (site access, electrical power, water). Using the existing infrastructure and data gathered during previous studies at the site provided baseline information and allowed for relatively easy manipulations of the system's operating conditions.

The geology in the vicinity of the test site consists of unconsolidated alluvial deposits that have been deposited on the top of the undulatory erosional surface of the Navarro Clay (see Figure 3-3 Cross Section of Geology). The alluvial deposits consist of gravel, sand, silt, and clay, ranging in thickness from 20 to 40 ft. From the surface downward, the geology typically consists of 1 to 4 ft of black organic clay, 6 to 16 ft of tan silty, calcareous clay; and 4 to 20 ft of clayey limestone and chert gravel. The surface of the Navarro Clay is irregular and characterized by ridges and channel-like depressions.

Groundwater in the area of the demonstration site is primarily present in the limestone/chert layer. The water table is approximately 15 to 20 ft bgs, and the saturated thickness is from 5 to 12 ft. Generally, groundwater flow is to the southwest with a flow velocity of approximately 0.3 ft/day. The regional water table gradient is approximately 0.003.

Volatile organic compounds (VOCs) in the site groundwater consist primarily of PCE, TCE, and their degradation products *cis*-DCE and VC. Total chlorinated ethene concentrations in the groundwater exceed 8,000 µg/L.

3.4 Predemonstration Testing and Analysis

3.4.1 NAS Fallon. Numerous site characterization and remedial activities have been performed and reports have been prepared describing the geologic, hydrogeologic, geochemical, and contaminant profile at the site. These reports include:

- Remedial Investigation Report Site 1 — ORNL 1994
- Site 1 Pumping Test Data Analysis and Interpretation at NAS Fallon, Nevada. — Battelle 1995a
- Final Report for Full-Scale Bioslurper Studies at Site 1, NAS Fallon, Nevada. — Battelle 1995b

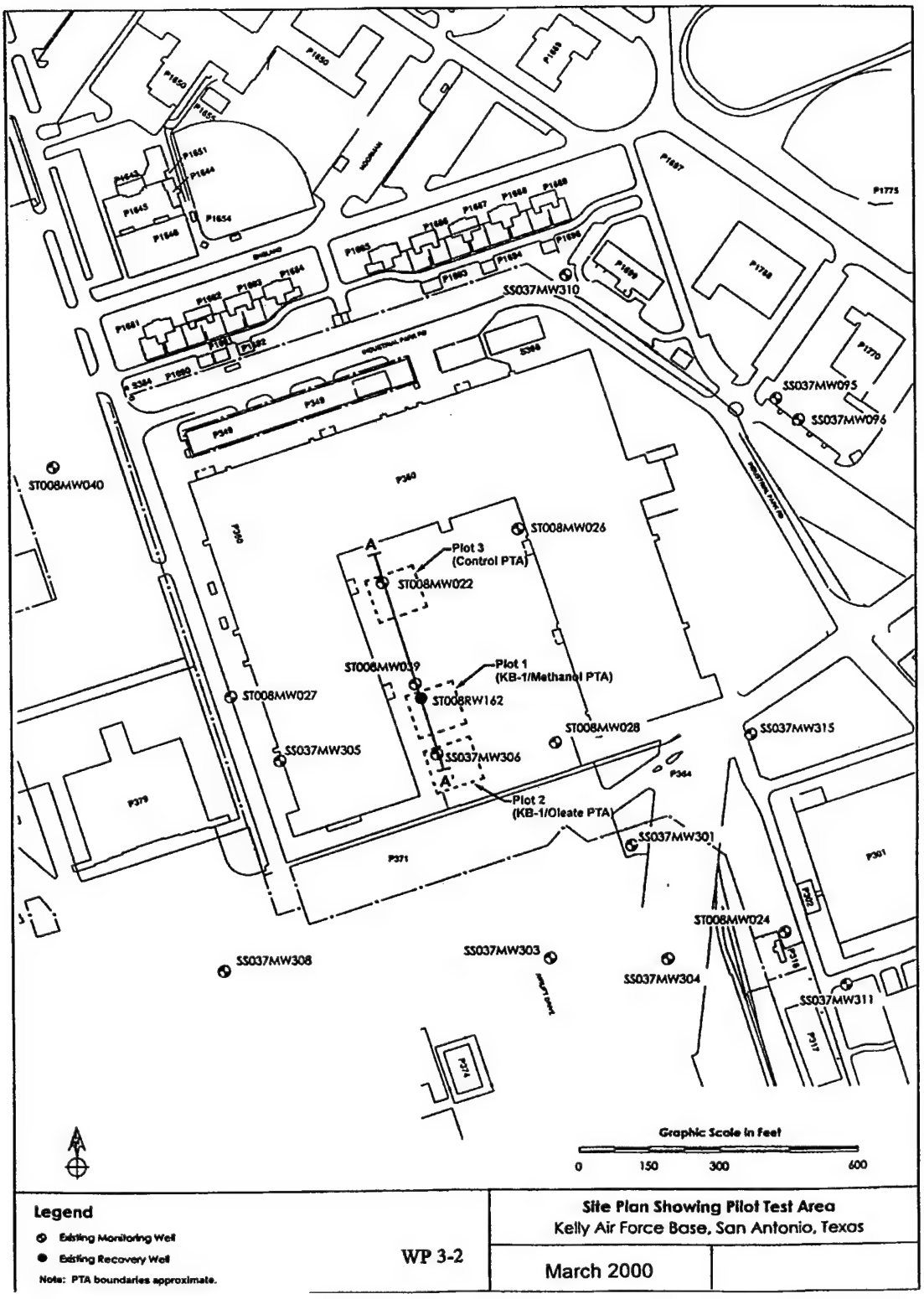

Figure 3-2. Map of Building 360 Site

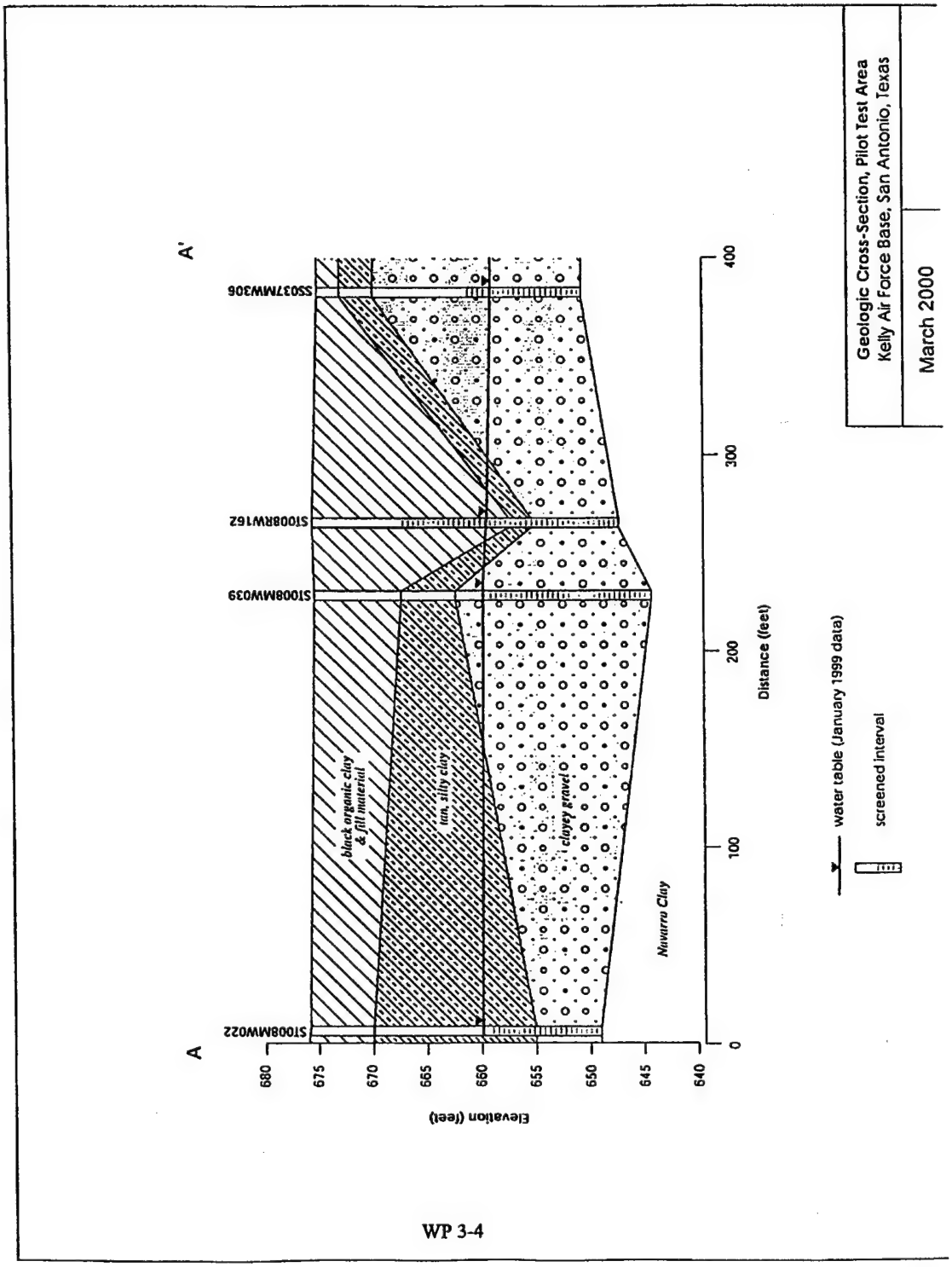

Figure 3-3. Cross Section of Surface Geology at the Building 360 Site

- Work Plan for Assessing the Feasibility of Intrinsic Remediation at Installation Restoration Program Sites, NAS Fallon, Nevada. — ORNL 1996

- Work Plan for In Situ Anaerobic Dechlorination of Chlorinated Solvents at NAS Fallon, Nevada. — Battelle 1996

- Comparison of Groundwater Alternatives Report, Phase I–Development of Potential Remedial Alternatives (Draft), NAS Fallon, Nevada. — ORNL 1997

The majority of these studies and reports focus on the characterization of the contaminant plume and the migration of the contaminants at Site 1. However, a couple of studies examined the possibility of achieving reductive dechlorination under natural conditions (ORNL, 1996) and through biostimulation (Battelle, 1996).

Laboratory studies performed by Battelle to assess dechlorination under natural conditions were performed in 1997. The data indicated that reductive dechlorination was not occurring under natural conditions, and it was suggested that the high sulfate concentrations in the groundwater from the site might be inhibiting the dechlorination process. Barium chloride was added to the microcosm bottles to reduce the sulfate concentrations through a precipitation reaction. Although the sulfate concentrations were significantly reduced in the test bottles, complete dechlorination was never observed.

Microcosm studies were conducted by the Air Force, Cornell University, and Battelle to investigate biostimulation as a means of achieving complete reductive dechlorination. The studies indicated that dechlorination to ethene was not achieved through biostimulation alone.

3.4.2 Kelly AFB. Testing of the bioaugmentation technology previously was conducted at the Building 360 site. This project was conducted by RTDF, and the effort was led by GeoSyntec, Inc. with the objective of successfully demonstrating the bioaugmentation technology for chloroethene remediation. KB-1 culture was injected into the test plot to test the technology. A description of this demonstration is included with the description and results of the Environmental Security Technology Certification Program (ESTCP)-funded projects because the ESTCP project, at Kelly AFB was conducted as a follow on to the RTDF demonstration.

3.5 Testing and Evaluation

3.5.1 Demonstration Installation and Startup. Details of the setup, operation, and results of the microcosm testing performed with the NAS Fallon soils are presented in the microcosm report prepared for ESTCP (included as Appendix D f this report). No further discussion of the work at NAS Fallon is provided in the main part of this document.

Following the failure to achieve complete biological dechlorination in the Fallon microcosm tests, the ESTCP bioaugmentation project was moved to Kelly AFB with new objectives. The testing at Kelly AFB was a continuation of work conducted by RTDF and Geosyntec, and Test

Plots 1 (treatment plot) and 3 (control plot) that were designed and used for RTDF/Geosyntec project were used for the ESTCP demonstration. Plan view and cross-sectional diagrams of the systems are displayed in Figures 3-4 and 3-5, respectively. Each plot has a total of nine wells: one injection well, three extraction wells, and five monitoring wells. Three of the monitoring wells are aligned along the center of the plot parallel to the groundwater flow direction and located at a distance of 8, 12, and 22 ft downgradient of the injection well. The other two monitoring wells are aligned perpendicular to groundwater flow and were initially installed to be outside the zone of influence of the system. Each of the wells in both plots are completed to a depth of 25 ft bgs and were screened from 15 to 25 ft (below the water table) to reduce the opportunity for aeration and increased oxygen concentrations of the groundwater as it moved through the treatment system.

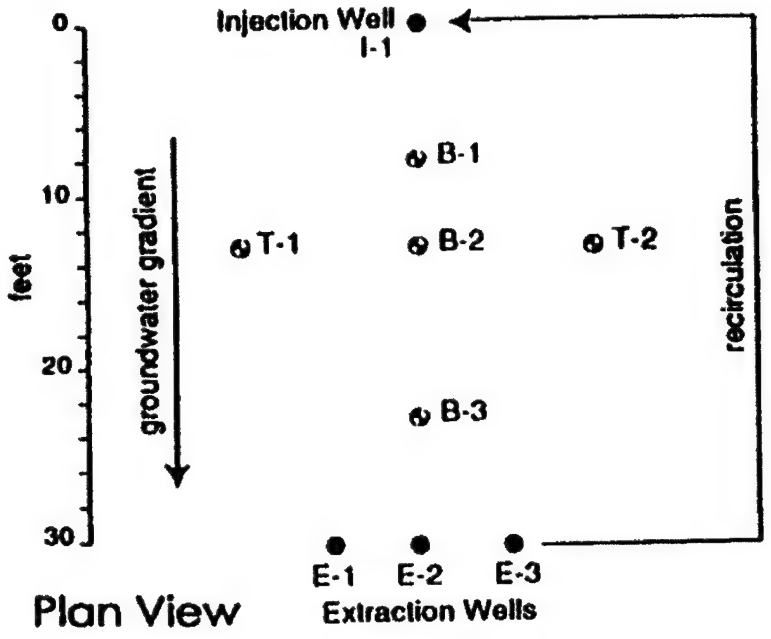

Figure 3-4. Plan View Diagram of the Test Plot at the Building 360 Site, Kelly AFB, Texas

An injection/extraction process was used to hydraulically isolate the test and control plots. Groundwater was removed from the extraction well and recirculated through the system by injecting it into the injection well. The injection/extraction rates were the same as those used during the RTDF/Geosyntec project (approximately 2 gallons per minute [gpm]). These injection/ extraction rates were calculated by GeoSyntec using a groundwater modeling program and were demonstrated to have adequate isolation of the test cells and allow for a reasonable residence time in the cells during the RTDF/GeoSyntec project. Groundwater was extracted from the extraction wells using Grundfos submersible pumps and injected into the injection well after the addition of the amendments (electron donor, nutrients, etc.). The groundwater was pumped through a mobile shed where the nutrients were injected into the water stream using piston-style metering pumps.

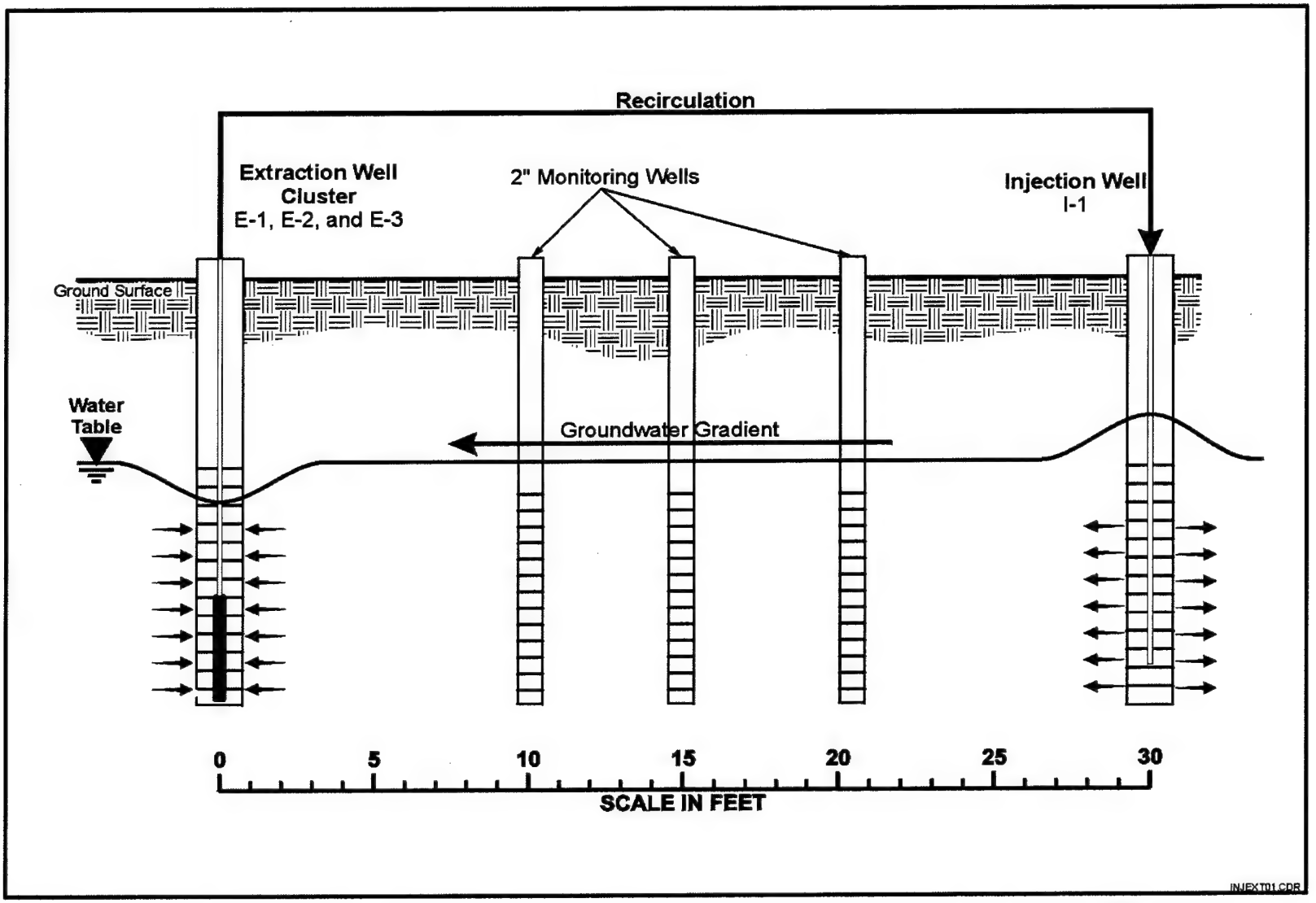

Figure 3-5. Cross-Sectional View Diagram of the Test Plots at the Building 360 Site, Kelly AFB, Texas

3.5.2 Period of Operation. The test was conducted in three phases to examine the effectiveness and robustness of the bioaugmentation technology. Phase I was conducted under the RTDF/GeoSyntec project and the data were used to demonstrate the feasibility of the bioaugmentation technology. This phase of the demonstration was initiated on November 12, 1999 with the collection of baseline samples and groundwater recirculation. For the first 89 days, only bromide was added to the system to test the flow dynamics within the test cells. After 89 days of operation (February 9, 2000) and confirmation of the appropriate flow dynamics, acetate and methanol were added to the system. Both electron donors were added at time-weighted concentrations of 3.6mM. The addition of electron donors was continued until September 25, 2000 (319 days after the initiation of the system). On May 6, 2000 (Day 176), the KB-1 culture was injected into the test cell. Approximately 3.5 gallons of the culture was injected into the injection well using an argon purge system to maintain low oxygen levels in the treatment plot.

Phase II was a die-off study to examine the effect of depriving the system of an electron donating substrate on the population of the bioaugmentation culture and the dechlorination reactions. The RTDF/GeoSyntec project concluded on September 25, 2000, and the addition electron donor and recirculation of groundwater was stopped at that time. Approximately 332 days after the end of the RTDF/GeoSyntec project (August 23, 2001), groundwater samples were collected from the test plot for chemical and microbial analyses. The results of these analyses served to determine the effects of electron donor depletion on the microbial populations and the dechlorination process. In addition, these samples provide a baseline for the Phase II and Phase III portions of the demonstration. On October 15, 2001 the addition of the methanol and acetate were initiated again.

Phase II of the demonstration continued until March 9, 2002 when it was believed that the dechlorination process had reached near-steady-state conditions and sulfate was added to the system to test the robustness of the culture. Initially, sulfate was added to the system at a molar level equivalent to the addition of the methanol. After apparently no effect on the dechlorination process at the initial sulfate injection levels, the sulfate injection levels were increased to a molar equivalent level to the addition of both the methanol and the acetate on May 9, 2002. Phase III continued with the injection of sulfate until July 19, 2002 when the base no longer wished to continue the bioaugmentation activities because another remediation project was to be performed at that site later in the year. Near the end of July, heavy rains fell at the site and it was believed that any electron donor, sulfate, and perhaps KB-1 culture in the test plot would have washed or diluted out of the system.

3.5.3 Amount/Treatment Rate of Material to be Treated. The system used during the demonstration was designed to test the feasibility of the technology for treating chloroethene-contaminated groundwater, and system was constructed to treat a defined volume of the aquifer. During the demonstration approximately 40,000 gallons of water was treated; converting all of the PCE to ethene.

3.5.4 Residuals Handling. No treated residual groundwater or soil was produced during this study because the system was designed as a recirculating system. There were no residuals handling issues.

3.5.5 Operating Parameters for the Technology. Both the test and control systems were operated on a continuous basis except for the "die-off phase" when no electron donor was added to the systems. Water was continuously recirculated through the plots with the addition of the electron donor occurring outside the treatment plots. The groundwater recirculation rate was maintained at approximately 2 gpm throughout most of the test. During periods when the screens on the injection wells would become fouled, the groundwater rates would be reduced to prevent overflow of the wells. Generally, fouling of the wells would be limited to about two weeks (until the wells could be redeveloped). This style of operation required that regular monitoring of the electron donor concentrations and injection rates, groundwater recirculation rates and groundwater levels in the plots. The electron donor concentrations and the fluid flow rates were monitored to keep the electron donor levels in the treatment and control plots constant. The target electron donor rate was 3.6 Mm, and the feed rate of the electron donor was adjusted to account for fluctuation in the water-injection rate. The water levels in the injection and extraction wells were monitored on a daily basis to investigate the occurrence of biological on mineral fouling of the wells.

3.5.6 Experimental Design. The demonstration was designed to evaluate the capability of the bioaugmentation technology and test the robustness of the KB-1 culture used at Kelly AFB. To evaluate the capability of the technology, the reductive dechlorination process was compared in a test plot that was augmented with the KB-1 culture with a control plot. Both plots were supplied the same electron donors at the same rates and the all other conditions were kept the same between the two plots.

To test the robustness of the culture the supply of the electron donor was stopped for both plots after injecting it for approximately 7 months. The period on no electron donor addition lasted for approximately 1 year, when the plots were sampled for dechlorination products and the presence of *D. ethenogenes*.

When it was determined that the *D. ethenogenes* was present and the reductive dechlorination process had reached near steady state conditions, sulfate was added to the system to determine at what sulfate levels the reductive dechlorination process would be inhibited. Sulfate was initially added at a molar equivalent level of the methanol added to the system. After approximately 2 months of lower-level sulfate addition, the sulfate injection levels were increased to a molar equivalent of both the methanol and acetate injection concentrations.

Throughout all phases of the studies attempts were made to keep the extraction and injection rates of the groundwater the same. However, due to fouling of the injection well, precipitation events, and problems with the pumps, this was not always possible. Electron donor and sulfate additions into the groundwater stream were constant through the use of metering pumps.

3.5.7 Sampling Effort. Groundwater samples were collected throughout all phases of the demonstration to evaluate the performance of the bioaugmentation technology at the Kelly AFB site. The samples were analyzed both in the field and in the laboratory, depending on the specific parameter being measured (Table 3-2). Table 3-3 displays the sampling schedule used during the demonstration. Groundwater samples were collected prior to starting the system to obtain baseline analyses. These samples were analyzed at a laboratory for PCE, TCE, DCE, VC, ethene, ethane, methane, volatile fatty acids (VFAs), bromide, nitrate, nitrite, and sulfate. In

addition, field monitoring was performed on the groundwater for bromide, dissolved oxygen, pH, conductivity, and reduced iron. Following the startup of the system, groundwater samples were collected to measure the effects of the experimental parameters that were adjusted. These samples also were analyzed for chloroethenes, ethene, ethane, methane, VFAs, bromide, nitrate, nitrite, and sulfate. Table 3-2 presents the analyte, method of analysis, analysis instrument, and location of analysis.

Table 3-2. Analytical Methods

Measurement	Method	Instrumentation	Analysis Location
Critical Measurements			
PCE, TCE, *cis*-DCE, VC,	U.S. EPA SW-846 Method 8260B	Gas Chromatograph/ Flame Ionization Detector-Electron Capture Detector (GC/FID-ECD)	Laboratory
Ethene, Ethane, and Methane	U.S. EPA (SOP)	GC FID	Laboratory
Volatile Fatty Acids (electron donor)	U.S. EPA (SOP)	GC/FID	Laboratory
Sulfate	U.S. EPA Method 300	Ion Chromatograph/ Conductivity Detector	
Bromide	U.S. EPA Method 300	Ion Chromatograph/ Conductivity Detector	Laboratory
Noncritical Measurements			
Nitrate, Nitrite, and Sulfate	U.S. EPA Method 300	Ion Chromatography/ Conductivity Detector	Laboratory
Bromide	Direct Reading	Bromide-Specific Electrode	Field
Dissolved Oxygen	Direct Reading	DO Probe	Field
pH	Direct Reading	pH Probe	Field
Conductivity	Direct Reading	Conductivity Meter	Field
Fe^{+2}	Hach Test Kit	Colorimeter	Field

SOP = Standard Operating Procedure.

In addition to the groundwater samples that were collected for monitoring the performance of the technology, groundwater samples were collected to monitor the transport and survivability of the microbial culture through the test cells. These samples were collected prior to the start of the test to obtain baseline conditions. After the initiation of the demonstration, additional samples were collected to evaluate the migration and survivability of the microbial population during the test. The samples were sent to Dupont, for analysis using ribosomal ribonucleic acid (RNA) analysis to detect the culture.

Table 3-3. Summary of Sampling Events for the Bioaugmentation Demonstration

Phase	Date	Wells Sampled	Analyses Performed	Microbial Analyses
Phase I	Nov. 12, 1999	B1-3, E1-3, T1-2, I1	Chloroethenes, Field Parameters, VFAs	No
	Feb. 9, 2000	B1	Chloroethenes, Field Parameters, VFAs	No
	Feb. 15, 2000	B1-3, E1-2, T1	Chloroethenes, Field Parameters, VFAs	No
	March 16, 2000	B1-3, E1-2, T1	Chloroethenes, Field Parameters, VFAs	No
	May 3, 2000	B1-3, E1-2, T1-2,	Chloroethenes, Field Parameters, VFAs	Yes
	May 22, 2000	B1-3, E1-2, T1-2	Chloroethenes, Field Parameters, VFAs	Yes
	June 5, 2000	B1-3, E1-2, T1-2	Chloroethenes, Field Parameters, VFAs	Yes
	June 27, 2000	B1-3, E1-2, T1-2	Chloroethenes, Field Parameters, VFAs	Yes
	July 17, 2000	B1-3, E1-2, T1-2	Chloroethenes, Field Parameters, VFAs	Yes
	Aug. 7, 2000	B1-3, E1-2, T1-2	Field Parameters Chloroethenes, VFAs	Yes
	Aug. 29, 2000	B1-3, E1-2, T1-2	Chloroethenes, Field Parameters, VFAs	Yes
	Sept. 25, 2000	B1-3, E1-2, T1-2	Chloroethenes, Field Parameters, VFAs	Yes
Phase II	Aug. 23, 2001	B1-6, E2, E5, T1, I1-2	Chloroethenes, Field Parameters, VFAs	Yes
Phase III	Oct. 11, 2001	B1-6, T1, E2, E5, I1-2	Chloroethenes, Field Parameters, Sulfate	No
	Nov. 7, 2001	B1-6, T1, E2, E5, I1-2	Chloroethenes, Field Parameters, Sulfate, VFAs	No
	Nov. 28, 2001	B1-6, T1, E2, E5, I1-2	Chloroethenes, Field Parameters, Sulfate, VFAs	Yes
	Dec. 19, 2001	B1-6, T1-2, E2, E5, I1-2	Chloroethenes, Field Parameters, Sulfate, VFAs	No
	March 2, 2002	B1, B3, I1	Chloroethenes, Field Parameters, Sulfate, VFAs	No
	April 25, 2002	B1-6, T1-2, E2, E5, I1-2	Chloroethenes, Field Parameters, Sulfate, VFAs	Yes

3.5.8 Sample Collection Procedures. Groundwater samples were routinely collected from the injection, extraction, and monitoring wells at both the control and tests plots at Kelly

AFB. A peristaltic pump was used to purge 3 well volumes of water out of each well. The purged groundwater was passed through an inline flow through cell and then into a waste container. While the water was being purged, a Horiba U22 Water Quality Meter was placed inside the flow-through cell and was used to measure the pH, conductivity, turbidity, dissolved oxygen, temperature, salinity, and oxidation-reduction potential (ORP) of the water. Once the purged water was removed from the well, the appropriate bottles were used to collect the samples of water. The VOC samples were preserved with hydrochloric acid (HCl), and the samples were then packed with ice in a cooler and shipped to Alpha Analytical Laboratories for analyses. A complete list of analyses, standard methods, hold times, and location of analysis is presented in Table 3-2.

3.5.9 Demobilization. The extraction pumps, metering pumps, mixing tanks and delivery tubing were removed from the wells and the control and test plots. In addition, the equipment trailers were returned to the rental companies. The injection, extraction, and monitoring wells that were installed during the RTDF/GeoSyntec portion of testing were left in place for any future testing to that may be performed at the site.

3.6 Selection of Analytical/Testing Methods

The groundwater samples were analyzed primarily for PCE and its degradation daughter products (TCE, *cis*-DCE, vinyl chloride, and ethene). In addition to the chlorinated ethene analyses, samples were collected for analysis of geochemical parameters (e.g., pH, ferrous iron, sulfate), and VFAs. Temperature, pH, conductivity, bromide, and dissolved oxygen (DO) were analyzed immediately at the site using an appropriate probe or hand-held meter. These analyses were selected because they provide the information required to determine the effectiveness of the technology.

3.7 Selection of Analytical/Testing Laboratory

The analyses performed on the groundwater samples are routine analyses and could be performed by many analytical service laboratories; therefore, no special capabilities were required for the laboratory to perform the analyses. The samples collected during the bioaugmentation demonstration were analyzed by:

Alpha Analytical
255 Glendale Ave.
Suite 21
Sparks, NV 89431

The gene probe analyses for the *D. ethenogenes* were performed at Dupont's laboratory, as KB-1 was their proprietary culture. Although KB-1 is a culture including a number of microorganisms, it is widely believed that *D. ethenogenes* performs the reductive dechlorination. Therefore, the gene probe analyses measured the presence of *D. ethenogenes* in the samples, and its presence was used to evaluate the robustness of the KB-1 culture.

4. Performance Assessment

4.1 Performance Criteria

A number of criteria were used to determine the success of the demonstration and the applicability of the bioaugmentation technology in the future and at other sites. Each of the parameters listed in Table 4-1 were qualitatively or quantitatively evaluated.

Table 4-1. Performance Criteria Used During the Bioaugmentation Demonstration

Performance Criteria	Description	Primary or Secondary
Contaminant Reduction	This technology is designed to reduce chloroethene contamination through sequential dechlorination to produce ethene as a final product.	Primary
Hazardous Materials	If successfully conducted, no hazardous materials would remain or be introduced through the implementation of the bioaugmentation technology. However, the use of bioaugmentation may prevent the formation and accumulation of more hazardous compounds, such as vinyl chloride that may be produced during biostimulation.	Primary
Factors Affecting Technology Performance	The bioaugmentation technology is affected by groundwater geochemistry, hydrogeologic characteristics of the site, and survivability of the culture. **Geochemistry:** Sulfate inhibits the reductive dechlorination process. High or low pH, high salinity or high levels of metals may adversely affect the introduced culture. **Survivability of the Culture:** Competition of the culture with the indigenous microbial population may affect the survival rate of the applied culture. Moderately alkaline conditions may favor the survival of the culture. Factors affecting the performance of the technology are discussed in greater depth in the Current State of the Bioaugmentation Technology.	Primary
Reliability	The bioaugmentation technology as it was applied during the demonstration was relatively reliable. Problems were encountered with the recirculation pumps. However, this style of pumping would be eliminated during full-scale operation	Secondary
Ease of Use	Both at the demonstration-scale and with full-scale operation the technology is relatively easy to use. The only pieces of equipment that are used are pumps for the injection of electron donor.	Secondary
Versatility	This technology is likely very versatile depending on the culture applied and the target contaminant. Cultures have been produced to treat chloroethenes,	Primary

Table 4-1. Performance Criteria Used During the Bioaugmentation Demonstration (Continued)

Performance Criteria	Description	Primary or Secondary
	MTBE, petroleum hydrocarbons, and chlorinated methanes, and PCBs. The bioaugmentation technology has long been used in the wastewater treatment systems.	
Maintenance	Moderate maintenance was required for the technology demonstration. Daily monitoring of the system equipment and the water levels in the injection/extraction were required to ensure the injection well would not overflow and the water levels in the in the extraction well was not lowered beneath the top of the screen. Also, pumps, and electron donor solutions needed to be monitored to ensure continuous flow.	Secondary
Scale-Up Constraints	The widespread application of the culture represents the greatest challenge with the scale-up of the technology. Direct contact between the culture and the contaminant is imperative for success of the technology. As the culture is injected in a well, the contaminants are pushed in front of the microbial culture. Therefore, the use of an in situ biobarrier may be the most effective method to provide intimate contact between the contaminants and the culture.	Primary

The performance of the technology at Kelly AFB was evaluated by determining if complete dechlorination could be achieved through bioaugmentation. Further, bioaugmentation was compared to biostimulation by operating a non-augmented control plot at the site under the same conditions as the bioaugmentation plot.

Successful implementation of bioaugmentation eliminates the accumulation of hazardous materials. Incomplete dechlorination can result in the accumulation of hazardous byproducts, such as vinyl chloride; whereas, the successful implementation of bioaugmentation completes the degradation pathway to the final products of ethene/ethane.

Site conditions (geochemistry, hydrogeology, and indigenous micro fauna) generally are factors affecting the successful conduction of bioaugmentation. As discussed in Section 2.3, certain site conditions may help or hinder the successful implementation of bioaugmentation. During this demonstration, culture survivability under extended periods of no donor addition and under elevated sulfate levels were investigated to determine their effects on the success of the technology.

Reliability, ease-of-use, and maintenance were evaluated by reviewing the Operation and Maintenance (O&M) logs. When contractors performed daily visits to the site, the operating parameters and system condition were recorded. The primary issue of concern that limited the operational time and increased the maintenance requirements of the system was the fouling of the injection well with biological or mineral media.

4.2 Performance Confirmation Methods

The effectiveness of the bioaugmentation technology at achieving complete dechlorination was evaluated by comparing the results produced in the test plot to those generated from the operation of a control plot within the same plume. The operating conditions and electron donor addition were same for both the control and test plots. In addition, prior to the addition of the culture, the system was allowed to operate until steady-state conditions had been achieved.

As was done with the testing of the overall bioaugmentation technology, the effects of eliminating the electron donor and the addition of sulfate were examined with the comparison of the results in the test plot with those in the control plot. Steady-state conditions also were achieved prior to modifying the conditions (i.e., electron donor and sulfate addition) in the test plot. A total of 15 sampling events were conducted over the course of the bioaugmentation study at Kelly AFB. In general, the sampling events occurred just prior to and then shortly after making a modification to the system test conditions. Following the sampling events near the time of the modification, samples were collected about every month to investigate long-term effects of the system changes. During each sampling event, a complete suite of chemical analyses was performed to determine the effects of the system modifications. Also, specific analyses were performed (i.e., microbial gene probe) to confirm the presence of the *D. ethenogenes* in areas where complete dechlorination was occurring.

Table 4-2. Expected Performance and Performance Confirmation Methods

Performance Criteria	Expected Performance Metric (pre demo)	Performance Confirmation Method	Actual (post demo)
PRIMARY CRITERIA (Performance Objectives) (Qualitative)			
Faster Remediation	Achieve complete dechlorination and reduce remediation time	Monitor chloroethene concentrations in the test and control plots	The bioaugmented plot achieved complete dechlorination, while the control plot did not. Therefore, bioaugmentation would decrease remediation times relative to biostimulation and natural attenuation
Ease of Use	Minimal operator training required	Monitor labor requirements	Minimal operator training was required for continuous operation.

Table 4-2. Expected Performance and Performance Confirmation Methods (Continued)

Performance Criteria	Expected Performance Metric (pre demo)	Performance Confirmation Method	Actual (post demo)
PRIMARY CRITERIA (Performance Objectives) (Quantitative)			
Feed Stream - Electron donor injection rate	3.6 mM (time-weighted)	Calibrated metering pumps	Achieved accurate injection levels
- Contaminant concentration	Total chloroethene 10 µM	U.S. EPA Method 8260	Maintained good mass balance
Hazardous Materials - Generated	None	Analysis for VC	Vinyl chloride was detected as a transient species
Factors Affecting Performance - Geochemistry	Geochemical conditions may limit survival of culture and dechlorination process	Analyze geochemical conditions (various methods), chloroethene concentrations (U.S. EPA Method 8260) and microbial populations	Natural water chemistry did not inhibit culture growth, nor did it prevent reductive dechlorination. Limited amounts of added sulfate (3.6 mM) did not affect dechlorination.
- Survivability	Lack of electron donor may kill culture	Eliminated electron donor addition, and monitored VFAs and microbial populations	This elimination of the electron donor addition did not stop reductive dechlorination process nor did the *D. ethenogenes* die off
SECONDARY PERFORMANCE CRITERIA (Qualitative)			
Reliability	Limited shutdowns	Record Keeping	Moderate to high number of shutdowns due to pump failures, high groundwater levels, and fouling wells and tubing.
Versatility - Intermittent operation	Yes	Experience from demonstration operation	Intermittent operation did not negatively affect system operation
- Other applications	Yes		Technology may be used for other chlorinated species and MTBE depending on the culture applied

Table 4-2. Expected Performance and Performance Confirmation Methods (Continued)

Performance Criteria	Expected Performance Metric (pre demo)	Performance Confirmation Method	Actual (post demo)
Maintenance - Required	Regular changing of tubing, development of the injection well	Experience from demonstration operation	Regular replacement of the tubing was required and development of the injection well was performed, but fouling was still a problem.
Scale-Up Constraints - Distribution of Culture	Widespread distribution of culture would be required for large-scale application	Monitored migration of culture throughout demonstration	The culture was spread throughout the test plot relatively quickly due to the operation of the recirculation system. For large-scale application, the culture may need to be used in a biobarrier form to get intimate contact between the culture, electron donor, and contaminants

4.3 Data Analysis, Interpretation, and Evaluation

As mentioned in Section 4.2, the success of the bioaugmentation technology at achieving complete dechlorination, the robustness of the microbial culture from limiting the electron donor input, and the effects of an increase in sulfate concentration in the groundwater at the test plot on the dechlorination process were primarily evaluated by comparing the results achieved in the test plot to those achieved in the control plot. The chloroethene concentrations measured throughout the demonstration at Kelly AFB are presented in Figures 4-1 through 4-8. The data are presented for each well over the duration of the demonstration. These data provide the analytical basis for the effectiveness of the bioaugmentation technology for treating PCE- and TCE-contaminated sites, and for the robustness of the KB-1 culture. Chloroethene concentrations in the figures are presented in molar concentrations to aid in the total molecular balance of the chlorinated ethene species. The inclusion of a timeline displaying the modifications of system conditions on Figures 4-1 through 4-8 was not possible. Therefore, the dates for each modification are provided in Table 4-3.

Table 4-3. Modifications to the Test Plot

Type of Modification to the System	Date
Start of the System	November 12, 1999
Start Electron Donor Addition	February 9, 2000
Addition of Culture	May 6, 2000
Stop Electron Donor Addition	September 25, 2000
Die-Off Samples Collected	August 23, 2001
Restart Electron Donor Addition	October 15, 2001
Start Addition of Sulfate (3.6 mM)	May 9, 2002
Start Addition of Sulfate (7.2 mM)	July 19, 2002

The data indicate that, although more than 90% of the PCE had been removed from the system, the dechlorination process was stalled at *cis*-DCE prior to the addition of the culture on May 6, 2000. At that point, no vinyl chloride or ethene had been detected. In each well, *cis*-DCE was the predominant chloroethene species, and the parent compounds (PCE and TCE) were at very low concentrations, generally less than 1 μM. In general, most of the wells displayed good molar balance between the chloroethenes compounds. Typically, the total molar concentration was near 6 or 7 μM.

Following the addition of the KB-1 culture, the concentrations of *cis*-DCE decrease relatively rapidly. Within 115 days of the addition of the microbial culture, ethene was detected in many of the wells, and about that time also becomes the predominant compound within the test cell. In addition to the decrease in the *cis*-DCE concentrations, the PCE and TCE concentrations in the plot remain low. Also, just prior to the production of the ethene, there is a spike in the vinyl chloride concentrations. The changes in the chloroethene concentrations in the test plot and the absence of change in the control plot indicate that the KB-1 culture effectively produced complete dechlorination. Further, the gene probe data in Table 4-4 provide supporting data that addition of the KB-1 culture resulted in the complete dechlorination of the PCE and TCE. This table provides relative pixel PCR density as measured by NIH image 1.62. A value of three is the highest pixel density of *Dehalococcoides* sequence (>10^5 Dhc copies per reaction). A value of two would be a midrange pixel density of Dhc sequence (10^3 to 10^5 Dhc copies per reaction), and a value of one would be a low range pixel density (10^1 to 10^3). A pixel density of zero would indicate no Dhc was detected (<100 Dhc copies per reaction).

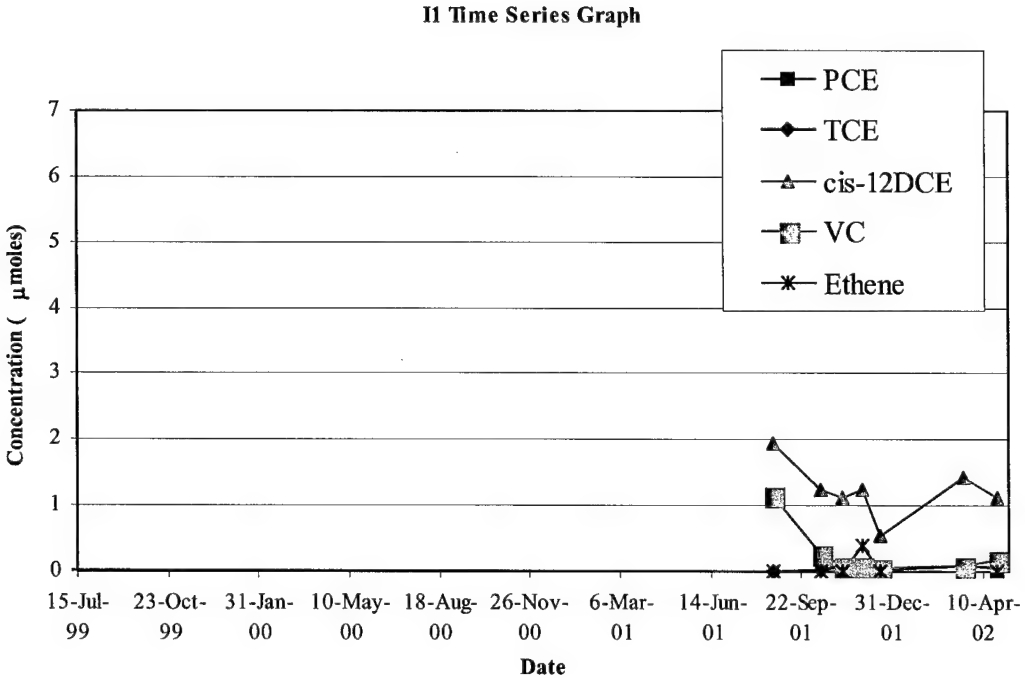

Figure 4-1. Chloroethene Concentrations in Well I1 (Test Plot, Injection Well

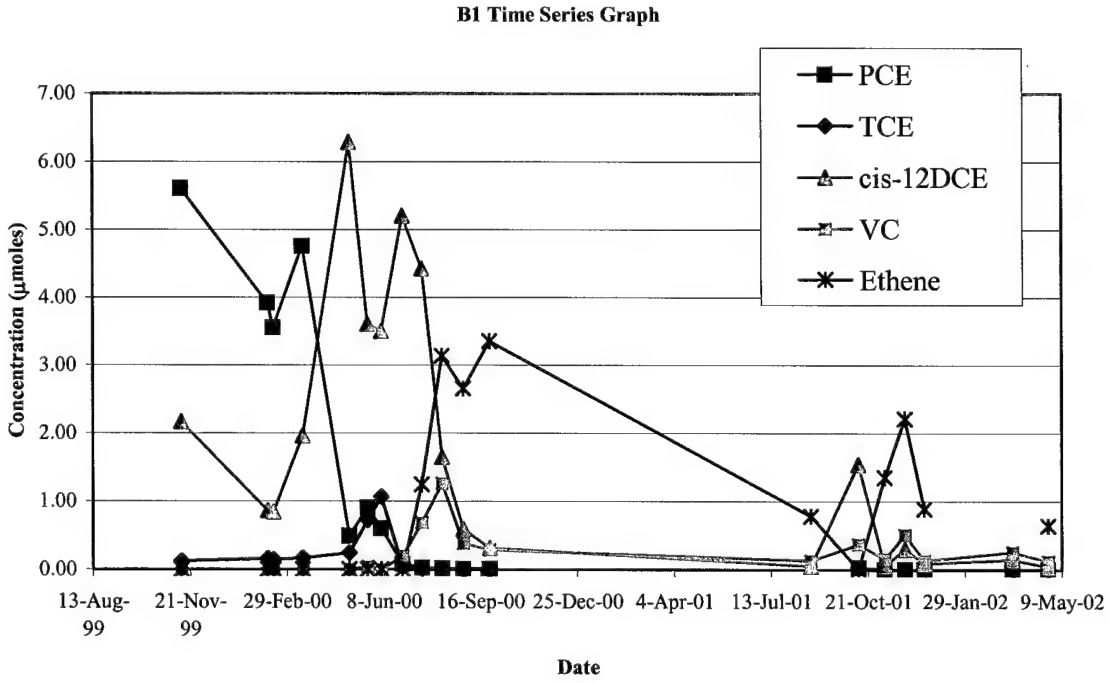

Figure 4-2. Chloroethene Concentrations in Well B1 (Test Plot, D = 8 ft from IW

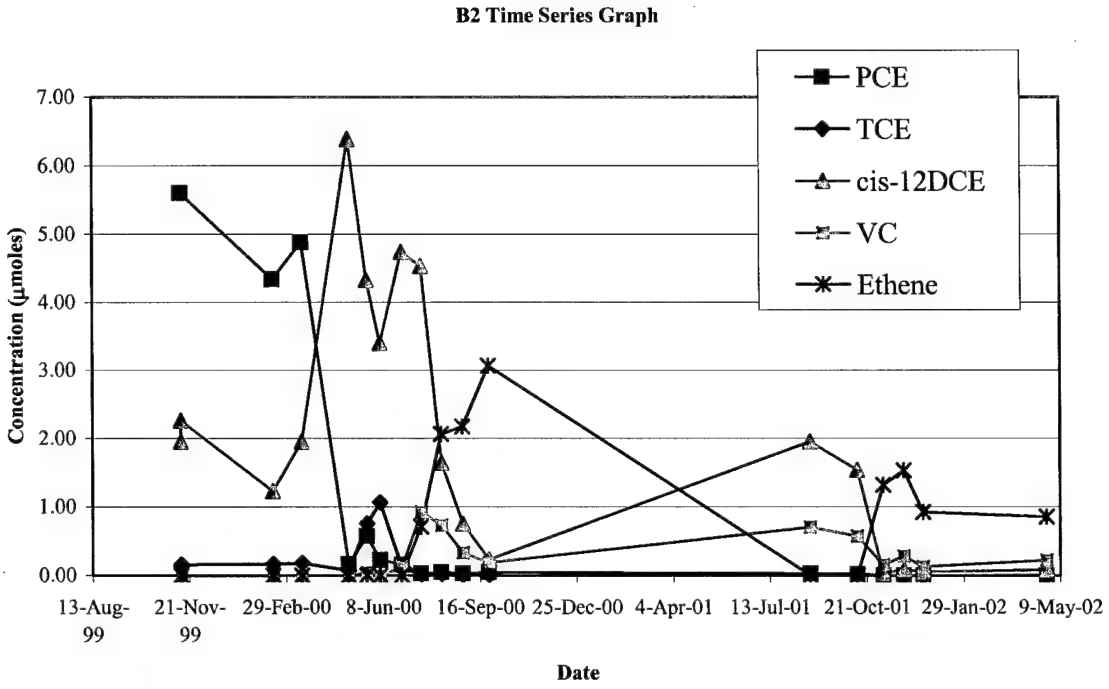

Figure 4-3. Chloroethene Concentrations in Well B2 (Test Plot, D = 12 ft from IW)

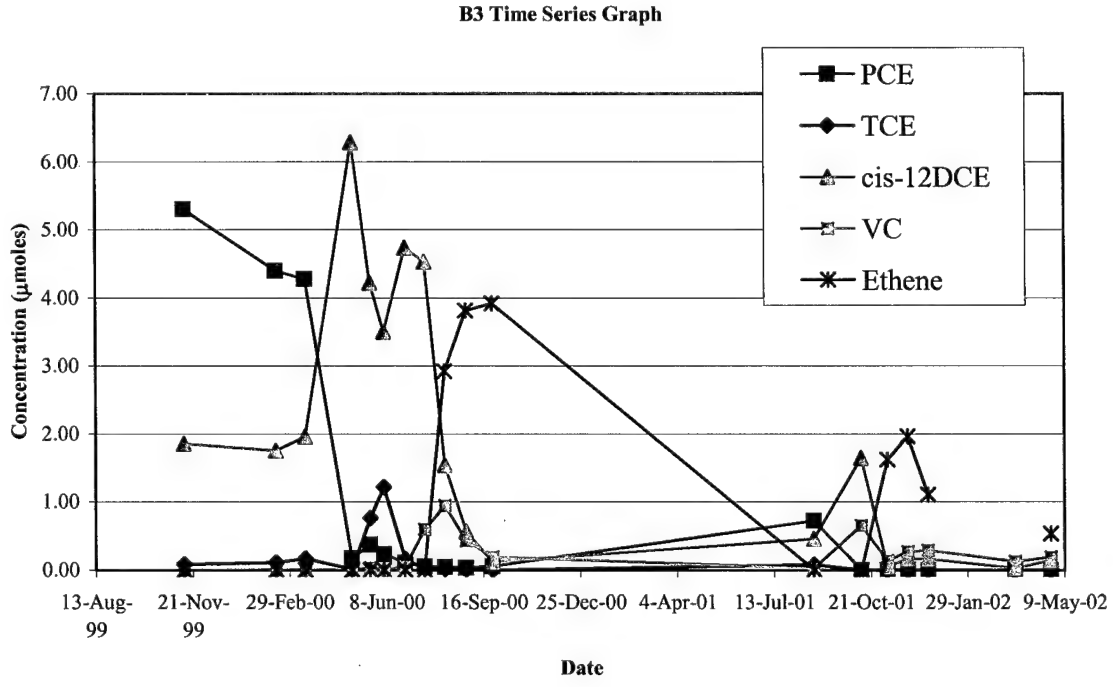

Figure 4-4. Chloroethene Concentrations in Well B3 (Test Plot, D = 22 ft from IW)

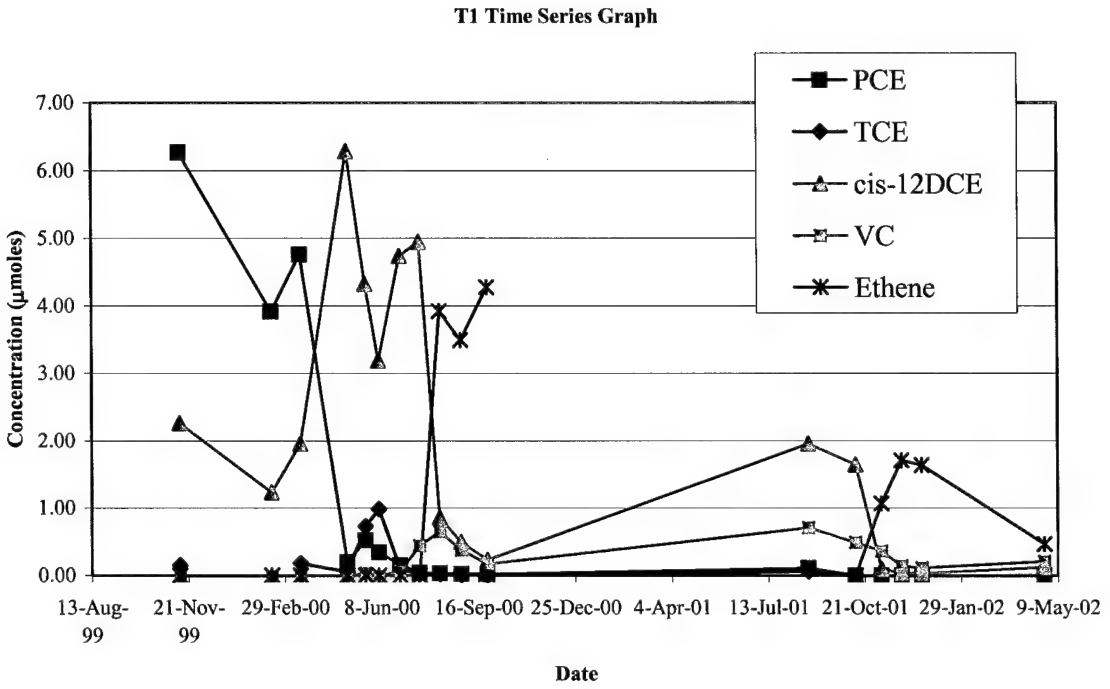

Figure 4-5. Chloroethene Concentrations in Well T1 (Test Plot, D = 12 ft from IW)

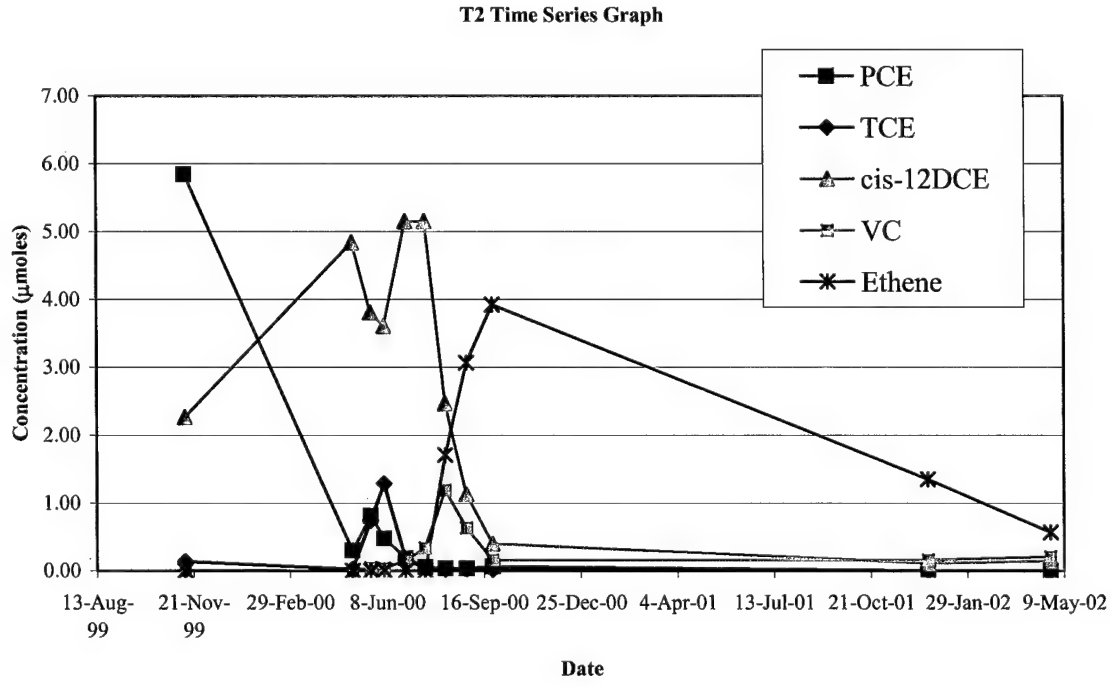

Figure 4-6. Chloroethene Concentrations in Well T2 (Test Plot, D = 12 ft from IW

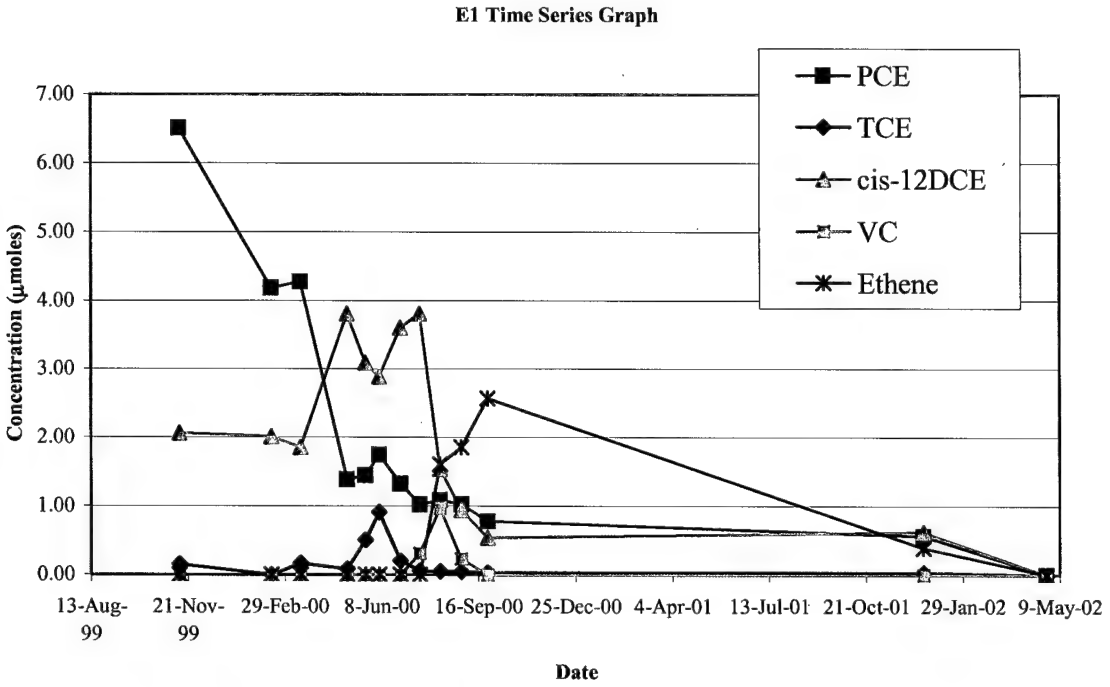

Figure 4-7. Chloroethene Concentrations in Well E1 (Test Plot, D = 30 ft from IW)

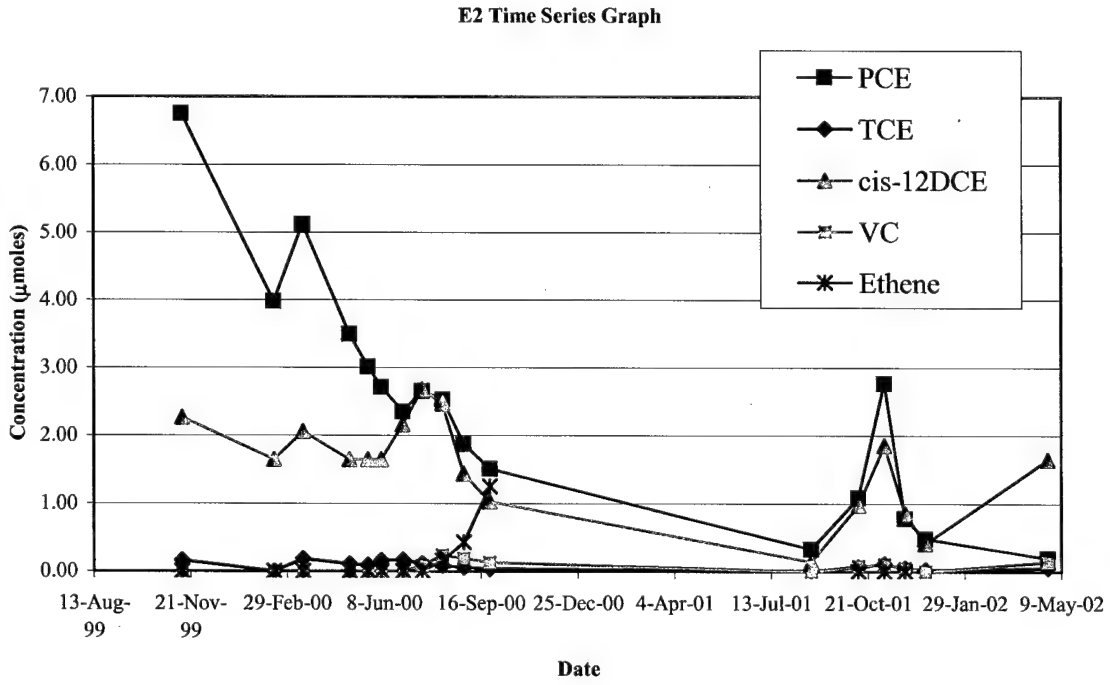

Figure 4-8. Chloroethene Concentrations in Well E2 (Test Plot, D = 30 ft from IW)

Table 4-4. Detection of *Dehalococcoides* Sequences in Test Plot after Bioaugmentation

Sample Date	Day	Monitoring Well Identification							
		IW	B1	B2	B3	T1	T2	E1	E2
5/3/00	173	0	0	0	NT	NT	NT	0	NT
5/6/00	176 (t =0)	1	0	NT	NT	NT	NT	0	NT
5/22/00	192	3	0	NT	NT	NT	NT	0	NT
6/5/00	206	3	1	NT	NT	NT	NT	0	NT
6/27/00	228	2	2	NT	NT	NT	NT	0	NT
7/17/00	248	1	3	2	1	NT	NT	0	NT
8/7/00	269	1	3	3	1	NT	NT	1	NT
8/29/00	291	1	3	3	2	2	2	2	1
9/24/00	318	1	3	3	2	3	2	2	1
8/24/01	650	2	NT	1	NT	NT	NT	NT	1

Following the successful demonstration of the bioaugmentation technology at promoting complete reductive dechlorination, the system was shut down and electron donor addition was stopped on September 25, 2000. The test plot was not supplied electron donor until October 15, 2001. During the period when no electron donor was being added, groundwater samples were collected on August 23, 2001, for microbial analyses to determine if *D. ethenogenes* could survive without the addition of electron donor. Also, chemical analyses were performed on the samples to evaluate if reductive dechlorination was still occurring. Data indicate that *D. ethenogenes* was still present after nearly one year without the addition of electron donor. No electron-donating substrate was detected in the VFA analyses that were performed. Although the *D. ethenogenes* appeared to be present across the entire test plot, complete reductive dechlorination (with the production and presence of ethene) was only occurring in well B1. On October 11, 2001 the analytical data demonstrate that the reductive dechlorination process was again limited to *cis*-DCE or VC (in some cases).

With the addition of the electron donor on October 15, 2001, complete dechlorination to ethene was observed relatively quickly. Within 23 days of the addition of the electron donor, ethene was detected in all but one of the wells that were sampled. At the same time, the concentrations of the chloroethenes (*cis*-DEC and vinyl chloride) decrease to near-zero concentrations.

After it was demonstrated that the culture was present and dechlorination was still occurring, the robustness of the culture was tested by adding sulfate to the test plot. On March 9, 2002, sulfate was added to the system at a concentration of 3.6 mM, which is equivalent to the molar addition of methanol to the system. Samples collected between March 9 and May 19, 2002 (when the sulfate addition was increased to 7.2mM), the relative concentrations of the chloroethene concentrations remained stable, indicating that the dechlorination process is not disturbed by the sulfate addition. Unfortunately, no groundwater samples could be collected after the May 19, 2002 increase in the sulfate addition.

The results of this study have demonstrated that the addition of KB-1 culture to the test plot at Kelly AFB promoted complete dechlorination. The molar distribution of PCE, TCE, and c-DCE was reduced from approximately 100% of the total chloroethenes at the beginning of the study to

less than 20% of the total chloroethenes at the end of the study. Inversely, the molar distribution of ethene increased from 0% at the beginning of the study to approximately 77% by the end of the study.

The reduction of PCE to ethene correlated well to the presence of the *D. ethenogenes*. The test data demonstrate that no reductive dechlorination past c-DCE occurred prior to the inoculation of the test plot with the KB-1 culture and the complete reductive dechlorination was not observed in any of the monitoring wells until the *D. ethenogenes* was detected. Further, the results demonstrated that the culture had populated the entire test plot within three months of the addition culture to the injection well, and the total population density of the *D. ethenogenes* were greater at the end of Phase I than were initially introduced with the inoculum.

The test data from Phase II indicate that the culture has a high survivability under the conditions of no electron donor. Following a period of approximately one year that the test plot was deprived of electron donor, analytical data indicate that complete reductive dechlorination was detected in one well and the KB-1 culture was present across the entire plot.

Phase III data suggest that KB-1 culture is relatively unaffected by the increase of sulfate concentration in the test plot groundwater. Following the addition sulfate to the test plot at molar equivalent of the methanol added to the system, the reductive dechlorination process was only slightly inhibited. Prior to the addition of sulfate at 3.6 mM, the distribution of the chloroethenes was 16.9, 0.9, 19.0, 8.9, and 54.3% for PCE, TCE, c-DCE, VC and ethene, respectively. Approximately six weeks following the addition of sulfate, the PCE, TCE, c-DCE, VC, and ethene concentration had only changed slightly to 3.9, 0.9, 32.9, 16.8, and 45.5%, respectively. However, the VFA analyses indicated the acetate was present following the addition of sulfate suggesting that the use of the electron was affected by the increase in the groundwater sulfate concentration. Unfortunately, no samples could be collected after the increase in the sulfate addition (to 7.2 mM).

Throughout the demonstration, the data from the test plot were compared to the control plot, which was operated under the same conditions as the test plot (aside from the addition of the KB-1 culture). The data from the control plot indicate that complete dechlorination was not achieved through biostimulation (Appendix B). At the start of the project the distribution of PCE, TCE, c-DCE, VC, and ethene were similar to those found in the test plot (approximately 60% PCE, 2% TCE, and 38% c-DCE). Following the addition of the electron donor for 10 months the distribution of PCE, TCE, and c-DCE were 37, 23, and 40%, respectively, and no VC or ethene was detected. However, strongly reducing conditions were produced in the control plot, which is evident from the increase in methane concentrations in the groundwater. The methane concentrations in the control plot increased from non-detect at the start of the test to 1.3 mg/L after 10 months of electron donor addition. During Phases II and III, VC was only detected in four samples at concentrations <2.1 µg/L.

5. Cost Assessment

The bioaugmentation demonstration was conducted in two stages: a microcosm test (performed at NAS Fallon), and a full-scale demonstration conducted at Kelly AFB. The microcosm testing at NAS Fallon indicated the bioaugmentation would not be feasible at that site. Previous testing had been conducted at Kelly AFB; therefore, no microcosm testing was required prior to full-scale work at that site. Because microcosm testing is recommended prior to performing a full-scale remediation project, this cost assessment includes costing for both the microcosm and full-scale stages of the demonstration.

5.1 Cost Reporting

Throughout the course of this demonstration, the cost data were tracked to provide accurate cost information on the scale-up of the technology once it had been demonstrated. Costs associated with labor, consumable equipment, capital equipment (rented and purchased), subcontracted labor (O&M providers), and purchased services (drillers and analytical). The system used at Kelly AFB generally was established prior to the ESTCP testing at the site; therefore, some of the costs had to be estimated for the field scale testing of the technology.

5.1.1 Microcosm Testing. The cost to perform microcosm testing with NAS Fallon soils was approximately $78,000. Table 5-1 shows the cost breakdown. During the microcosm testing, two conditions were tested: an unaugmented control and augmented test bottles. Both of these conditions were conducted in triplicate and at least biweekly analyses were performed on the bottles. The duration of the microcosm testing was 31 weeks. The soil samples were collected from an average depth of 15 ft bgs. Although GE provided the culture, an estimated cost of $500 was used for GE to produce the culture.

5.1.2 Field Testing. The cost to complete a field test of bioaugmentation at Kelly AFB is presented in Table 5-2. The total cost of performing a field test of the bioaugmentation technology was estimated at $255,936. Again, some of the costs associated with installation and the cost of the culture had to be estimated because the system had been used previously for bioremediation testing.

The layout of Kelly AFB consists of one injection well, three extraction wells, and six monitoring wells covering an area of approximately 30 feet by 20 feet. The total volume of groundwater treated by the demonstration system was approximately 40,000 gallons. Monitoring wells used for the demonstration were constructed of 2-inch PVC, and the injection and extraction wells were 4-inch PVC. The field trailers were used to store equipment and provided a location for the electron donor, tracer, and sulfate to be added to the system.

Mobilization costs included transporting the field trailers to the site and securing the trailers at the site. The majority of the site costs include the construction costs for preparing the site, such as drilling and electrical installation. The labor and analytical costs are the dominant part of the variable costs, where the equipment and materials costs are much lower.

Table 5-1. Estimated Cost of Microcosm Testing

Activity	Unit Cost	Quantity	Cost
Microcosm Test Plan	$5 K	1	$5K
Microcosm Testing			
Soil Collection			
Labor	$2K	1	$2K
Travel	$3K	1	$3K
Drilling costs			
Mobilization	$1K	1	$1K
Drilling (20-ft deep)	$25/lf	100 lf	$2.5K
Waste disposal	$2K	1	$2K
Misc. (decontamination, etc.)	$1K	1	$1K
Consumables and supplies	$1K	1	$1K
Dechlorinating culture	$0.5K	1	$0.5K
Conduct Testing			
Labor	15K	1	$15K
Analytical services			
VOCs	$100/sample	200	$20K
Data analysis	$5K	1	$5K
Reporting	$10K	1	$10K
Total Cost for Microcosm Testing			**$78K**

5.2 Cost Analysis

5.2.1 Cost Comparison. A typical technology for treating chlorinated solvent-contaminated sites is pump-and-treat. For full-scale operation of bioaugmentation, the use of a biobarrier would likely provide the most effective method of aquifer remediation. The use of a biobarrier is a relatively simple and inexpensive remediation method, which may also eliminate some of the problems associated with achieving adequate distribution of the culture. A comparison of the use of a biobarrier and pump-and-treat over time is provided in Table 5-3.

The costs presented in the cost comparison were derived from a generic site with a 5-acre chlorinated ethene plume with dimensions being 300 ft by 700 feet. The depth to groundwater is set as 15 ft and the total depth of the aquifer is 25 feet.

For construction of a biobarrier, it was believed that 20 wells would be required across the leading edge of the plume. Each of these wells would be screened across the thickness of the saturated zone. The biological culture would be injected into each of the wells, and the desired cell density (10^4 cells/mL) in the aquifer would be achieved through pumping and cell growth. It is estimated that approximately 25 L of the culture would need to be added to the system. The wells installed for the pump-and-treat system would be evenly spaced throughout the plume, and it was believed that 50 wells would be required to cover the plume.

Table 5-2. Costs for Field Demonstration at Kelly AFB, TX

Cost Category	Subcategory	Costs ($)
	FIXED COSTS	
1. CAPITAL COSTS	Mobilization/demobilization - Mobilization of trailers	 $1,000
	Demonstration Plan	$15,000
	Site work	$20,000
	Equipment Cost - Extraction/Metering Pumps - Manifold/Tubing	 $3,750 $600
	Installation - Drilling - Electrical	 $22,367 $5,000
		Subtotal $67,727
	VARIABLE COSTS	
2. OPERATION AND MAINTENANCE	Labor - Subcontractor - Battelle personnel	 $75,678 $20,312
	Materials and Consumables - Chemicals - Material	 $3,000 $5,000
	Travel costs	$9,250
	Culture Chemical/Biological Analyses	$10,000 $43,853
	Performance Data Analysis/Reporting	$11,454
	Trailer Rental	$9,600
		Subtotal $188,209
	TOTAL COSTS	
		TOTAL TECHNOLOGY COST : $255,936

The costs for equipment and materials are much higher for pump-and-treat primarily because of the costs of the air stripping and catalytic oxidizer systems. It was estimated that these components would be approximately $105,000. The only additional materials costs that bioaugmentation would have are with the biological culture (estimated at $15,000).

Table 5-3. Cost Comparison for Field Implementation of Bioaugmentation and Pump-and-Treat

Cost Category	Subcategory	Bioaugmentation Costs ($)	Pump-and-Treat Costs ($)
	FIXED COSTS		
1. CAPITAL COSTS	Mobilization/demobilization		
	- Mobilization of trailers	$1,000	$1,000
	Demonstration Plan	$25,000	$20,000
	Site work	$40,000	$100,000
	Equipment Cost		
	- Extraction/Metering Pumps	$6,000	$4,000
	- Manifold/Tubing	$1,000	$5,000
	- Treatment Equipment (Air Stripping/Catalytic Oxidizer)	$0	$105,000
	- Biological Culture	$15,000	$0
	Installation		
	- Drilling with Disposal	$33,000	$83,000
	- Electrical	$10,000	$60,000
	Subtotal	$131,000	$378,000
	VARIABLE COSTS		
2. OPERATION AND MAINTENANCE	Labor		
	- Subcontractor	$130,000	$390,000
	Materials and Consumables		
	- Chemicals	$40,000	$0
	- Material	$24,000	$75,000
	- Electricity	$5,000	$25,000
	- Propane	$0	$20,000
	Chemical/Biological Analyses	$55,000	$42,000
	Performance Data Analysis/Reporting	$11,000	$11,000
	Trailer Rental	$10,000	$10,000
	Subtotal	$275,000	$573,000
	TOTAL TECHNOLOGY COST :	$406,000	$951,000

The variable costs for pump-and-treat are significantly higher than those for bioaugmentation. While it is expected that the duration of the pump-and-treat system would be half as long as the biobarrier system, a significant cost associated with pump-and-treat is the operation and maintenance. It was estimated that the treatment systems for the pump-and-treat system would require 60 hours per/week while the bioaugmentation system would require 10 hours per week. The analytical costs associated with the biobarrier are only slightly higher due to the microbial analyses.

Although the cost comparison in this report was made between bioaugmentation and pump-and treat, a comparison may be made between bioaugmentation and biostimulation. However, a comparison between bioaugmentation and biostimulation is more difficult because the cost difference is not easily defined. The benefit from applying a culture results from a potential decrease in remediation time, and the magnitude of this decrease is uncertain as well as site dependent. Therefore, the cost benefit from applying the bioaugmentation technology over biostimulation is uncertain.

The cost of implementing bioaugmentation through the use of a biobarrier were also compared to the implementation of a permeable reactive barrier with iron medium. The cost associated with the permeable barrier were obtained from the cost and performance report for Evaluating the Longevity and Hydraulic Performance of Permeable Reactive Barriers at DoD sites (ESTCP, 2003). Costs for the permeable had to be estimated because unit costs were not presented in the report. It was assumed that the reactive barrier used during this cost estimate would need to be approximately 3 times as large as the barrier used during the field demonstration at NAS Moffet Field. The total cost of the sheet pile was estimated from the NAS Moffet Field installation. The NAS Moffet Field system was approximately 7.5 times narrower than the fictitious site used for these cost estimates. Because both technologies rely on natural groundwater movement, the treatment times for both the bioaugmentation and reactive barrier technologies were the same.

Table 5-4. Cost Comparison of Bioaugmentation and Permeable Reactive Barrier

Cost Category	Subcategory	Bioaugmentation Costs ($)	Permeable Barrier Costs ($)
	FIXED COSTS		
1. CAPITAL COSTS	Mobilization/demobilization		
	- Mobilization of trailers	$1,000	$1,000
	Work Plan	$25,000	$25,000
	Site work	$40,000	$100,000
	Equipment Cost		
	- Extraction/Metering Pumps	$6,000	$0
	- Manifold/Tubing	$1,000	$0
	- Biological Culture	$15,000	$0
	Installation		
	- Drilling with Disposal	$33,000	$0
	- Electrical	$10,000	$0
	- Sheet Pile Installation	$0	$405,000
	- Reactive Barrier/iron medium	$0	$417,000
	Subtotal	$131,000	$948,000
	VARIABLE COSTS		
2. OPERATION AND MAINTENANCE	Labor		
	- Subcontractor	$130,000	$40,000
	Materials and Consumables		
	- Chemicals	$40,000	$0
	- Materials	$24,000	$0
	- Electricity	$5,000	$0
	Chemical/Biological Analyses	$55,000	$40,000
	Performance Data Analysis/Reporting	$11,000	$11,000
	Trailer Rental	$10,000	$10,000
	Subtotal	$275,000	$101,000
	TOTAL TECHNOLOGY COST :	$406,000	$1,049,000

5.2.2 Cost Drivers and Potential Cost Impacts. The costs provided for each form of testing (i.e., microcosm or field test) were calculated under assumptions that were developed to describe a "typical" site. The actual costs for both microcosm testing and field testing depends on site-specific requirements/ logistics, so a cost comparison between the two approaches should be made during the process of selecting a test methodology. The variables that affect each approach and their potential impact are summarized in the following sections.

5.2.2.1 ***Cost Drivers.*** Depth to contamination is the single variable that could significantly impact the cost of conducting the microcosm tests. This variable has a direct effect on the costs associated with collecting the aquifer core material, specifically the drilling, waste disposal, and labor costs. The costs presented in Table 5-1 assume a depth of 15 ft. Collection of cores from shallower sites would be somewhat less expensive, while collection of soil from deeper sites would obviously be greater. For example, if the contamination were located at 200 ft, the total cost of the microcosm test would increase by $42,000. (The drilling costs would increase by $22,000 and the disposal costs would increase by $20,000.)

Similar to the microcosm approach, the depth of the contamination also affects the cost to perform field testing. However, the degree and extent of contamination also affect the costs. As the depth and size of the treatment area increase, the installation costs increase. Also, as the degree of contamination increases the mass of electron required increases, as well as the size of the equipment required to mix and deliver the additives.

5.2.2.2 ***Life Cycle Costs.*** For full-scale implementation of bioaugmentation, the capital costs and life-cycle costs are dependent on the design of the system used. As suggested previously, the most effective method of treating an aquifer with bioaugmentation likely would be a biobarrier. Capital costs for the installation of a biobarrier would be dependent on the depth of the aquifer and the lateral extent of contamination. Costs presented in Table 5-3 provide an estimate for the construction, operation and maintenance of a full-scale biobarrier. These costs were calculated for a 5-acre site with a plume length about 2 times the width.

Operational costs of a biobarrier would be relatively low due to the simplicity of the system. The bulk of operational costs would be associated with the regular sampling to ensure that the barrier is effectively treating the contaminated groundwater. Analysis would include chloroethene, dissolved gases, and VFA concentrations. The frequency of sampling and analyses likely would depend on the requirements of the overseeing regulatory agency.

Table 5-5 presents the life cycle costs for implementing the bioaugmentation technology in the biobarrier configuration and the reactive permeable barrier. For an operational period of 5 years, the total cost of the bioaugmentation technology would be $816,000 and the reactive barrier would be approximately $1,198,000. After 10 years of operation both technologies would be nearly the same at approximately $1,500,000. If the systems operate 20 years and the barrier material has a life of 10 years, the total cost of the bioaugmentation technology would be $2,871,000 and the reactive barrier would be 2,896,000.

Table 5-5. Present Value Estimates for the Bioaugmentation Technology in the Biobarrier Configuration and Reactive Barrier

Cost Scenario	Bioaugmentation	Reactive Barrier
Capital Investment Cost	$131,000	$948,000
Annual O&M Cost	137,000	50,000
Present Value over 5 years	816,000	1,198,000
Present Value over 10 years	1,501,000	1,448,000
Present Value over 20 years with 10 year life of barrier	2,871,000	2,896,000

Due to the relatively high cost for the installation and operation of the biobarrier system, it would be recommended that microcosm or field treatability testing be performed prior to the full-scale implementation of the technology. If complete dechlorination to ethene is not observed in the microcosm or field-scale testing, full-scale operation of the technology should not be performed. Performing on small-scale testing should significantly reduce the liability associated with the partial dechlorination of PCE/TCE to another regulated compound, such as vinyl chloride.

6. Implementation Issues

6.1 Environmental Checklist

The primary issues that need to be addressed with regulators deal with the injection of materials into the aquifer (i.e., microbial culture and electron donor). Because biostimulation is accepted in most states and regions, a permit for the addition of an electron donor should not difficult to obtain. An approval for the addition of a microbial culture may be more difficult to receive. Although bioaugmentation has been demonstrated at many locations, the addition of a bacterial culture may be new to some regulators.

6.2 Other Regulatory Issues

Discussions with regulators should be conducted by companies that traditionally have tested and implemented bioaugmentation, DoD facilities, and/or the U.S. EPA. A white paper on the state of the bioaugmentation technology has been prepared for this project. This document provides the state of the technology as well as future testing that needs to be performed and regulatory issues that need attention.

6.3 End-User Issues

With the data provided in this report, the Final Technical Report and the White Paper of the technology, the bioaugmentation technology has been successfully demonstrated for the remediation of chloroethenes at a single site. The RTDF is presently working to implement a full-scale anaerobic accelerated biodegradation system with bioaugmentation for remediation of a chlorinated solvent plume at Dover AFB, DE. As part of that effort, the RTDG will produce a series of reports outlining the appropriated application of and use of this technology. Data obtained through this study will provide crucial information relative to the overall effectiveness of accelerated anaerobic biodegradation and bioaugmentation for remediation of chlorinated solvents in ground water and will be incorporated into these documents. Screening criteria thusly will be identifies based on the demonstration projects will aid in the decision making process as to whether bioaugmentation is appropriate to pursue at contaminated sites.

7. References

Battelle, 1995a. Site 1 Pumping Test Data Analysis and Interpretation at NAS Fallon, Nevada. Prepared for NAS Fallon by Battelle.

Battelle, 1995. Final Report for Full-Scale Bioslurper Studies at Site 1, NAS Fallon, Nevada. Prepared for NAS Fallon by Battelle.

Battelle, 1996. Work Plan for In Situ Anaerobic Dechlorination of Chlorinated Solvents at NAS Fallon, Nevada. Prepared for NAS Fallon by Battelle.

Dybas, M.J., G.M. Tatara, W.F. Knoll, T.J. Mayotte, and C.S. Criddle, 1995. "Niche Adjustment for Bioaugmentation with *Pseudomonas* sp. Strain KC." In R.E. Hinchee, J. Fredrickson, and B.C. Alleman (Eds), *Bioaugmentation for Site Remediation*. Battelle Press, Columbus, OH. p. 77-84.

Jennings, D.A., J.N. Petersen, R.S. Skeen, B.M. Peyton, B.S. Hooker, D.L. Johnstone, and D.R. Yonge, 1995. "An Experimental Study of Microbial Transport in Porous Media." In R.E. Hinchee, J. Fredrickson, and B.C. Alleman (Eds), *Bioaugmentation for Site Remediation*. Battelle Press, Columbus, OH. p. 97-103.

ORNL, 1994. Remedial Investigation Report Site 1. Prepared for NAS Fallon by ORNL.

ORNL, 1996. Work Plan for Assessing the Feasibility of Intrinsic Remediation at Installation Restoration Program Sites, Naval Air Station Fallon, Nevada. Prepared for NAS Fallon by ORNL.

ORNL, 1997. Comparison of Groundwater Alternatives Report, Phase I–Development of Potential Remedial Alternatives (Draft), NAS Fallon, Nevada. Prepared for NAS Fallon by ORNL.

United States Environmental Protection Agency, 1996. *Drinking Water Regulations and Health Advisories*. EPA/822-3-96-002. Office of Water, Washington, D.C. October.

Witt, M.E., M.J. Dybas, R.M. Worden, and C.S. Criddle, 1999. "Motility-Enhanced Bioremediation of Carbon Tetrachloride-Contaminated Aquifer Sediments." *Environ. Sci. Technol.* 33(17):2958-2964.

Appendix A: Points of Contact

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Appendix B: Data Archive

KELLY AFB BIOAUGMENTATION PILOT TEST

Well	Date	Day	PCE		TCE		*cis*-12DCE		*trans*-12DCE		VC		Ethene		Ethane			
			µg/L	µM	µg/L	□□	µg/L	□□	µg/L	µM	µg/L	µM	µg/L	µM	µg/L	µM	µg/L	µM
B1 (MW136)	12-Nov-99	0	930	5.61	16	0.12	210	2.17	<10	-	<30	-	<2	-	NS		NS	
before acetate	9-Feb-00	89	650	3.92	21	0.16	84	0.87	<12	-	<30	-	<2	-	NS		NS	
	15-Feb-00	95	590	3.56	19	0.14	82	0.85	<12	-	<30	-	<2	-	NS		NS	
	16-Mar-00	125	790	4.76	22	0.17	190	1.96	<10	-	<10	-	<2	-	NS		NS	
ave of duplicates	3-May-00	173	81.5	0.49	32	0.24	610	6.29	0.65	0.01	0	0.00	<2	-	NS		NS	
	22-May-00	192	150	0.90	95	0.72	350	3.61	1.1	0.01	2.6	0.04	<2	-	NS		NS	
	5-Jun-00	206	99	0.60	140	1.07	340	3.51	1.7	0.02	<2	-	<2	-	NS		NS	
ave of duplicates	27-Jun-00	228	13	0.08	5.65	0.04	505	5.21	2.45	0.03	12.4	0.20	<2	-	NS		NS	
	17-Jul-00	248	3.9	0.02	1.2	0.01	430	4.44	2.6	0.03	43	0.69	35	1.25	NS		NS	
	7-Aug-00	269	2.6	0.02	<1.0	-	160	1.65	2.0	0.02	79	1.264	88	3.14	NS		NS	
ave of duplicates	29-Aug-00	291	1.45	0.01	-	0.00	57.5	0.59	1.65	0.02	24	0.38	74.5	2.66	NS		NS	
	25-Sep-00	318	1.5	0.01	-	0.00	31	0.32	1.4	0.01	18	0.29	94	3.35	NS		NS	
	23-Aug-01	650	<1.0	-	<1.0	-	4.5	0.05	NA	-	8.4	0.13	22	0.78	NS		NS	
	11-Oct-01	699	5.0	0.03	2.9	0.02	150	1.55	NA	-	23	0.37	<10	-	NS		NS	
	7-Nov-01	726	<1.0	-	<1.0	-	5.4	0.06	<1.0	-	9.9	0.16	38	1.35	NS		NS	
	28-Nov-01	747	<1	-	<1	-	29	0.30	1.3	0.01	32	0.51	62	2.21	NS		NS	
	18-Dec-01	767	<1.0	-	<1.0	-	8.8	0.09	<1.0	-	8.3	0.13	25	0.89	NS		NS	
	19-Mar-02	857	<1.0	-	<1.0	-	14	0.14	<1.0	-	16	0.26	NS	-	NS		NS	
	25-Apr-02	894	<1.0	-	<1.0	-	4	0.04	<1.0	-	7.7	0.12	18	0.64	<10	-	9,700	604.63
B2 (MW137)	12-Nov-99	0	930	5.61	18	0.14	190	1.96	<10	-	<30	-	<2	-	NS		NS	
	12-Nov-99	0	930	5.61	21	0.16	220	2.27	<10	-	<30	-	<2	-	NS		NS	
	15-Feb-00	95	720	4.34	22	0.17	120	1.24	<12	-	<30	-	<2	-	NS		NS	
	16-Mar-00	125	810	4.88	24	0.18	190	1.96	<10	-	<10	-	<2	-	NS		NS	
	3-May-00	173	28	0.17	10	0.08	620	6.40	< 1	-	< 1	-	<2	-	NS		NS	
	22-May-00	192	96	0.58	100	0.76	420	4.33	1.7	0.02	2.8	0.04	<2	-	NS		NS	
ave of duplicates	5-Jun-00	206	39	0.23	140	1.07	330	3.40	0.6	-	0	-	<2	-	NS		NS	
	27-Jun-00	228	27	0.16	15	0.11	460	4.75	2.2	0.02	8.1	0.13	<2	-	NS		NS	

KELLY AFB BIOAUGMENTATION PILOT TEST (Continued)

Well	Date	Day	PCE		TCE		*cis*-12DCE		*trans*-12DCE		VC		Ethene		Ethane			
	17-Jul-00	248	5.6	0.03	2.4	0.02	440	4.54	2.3	0.02	58	0.93	20	0.71	NS		NS	
	7-Aug-00	269	7.9	0.05	2.8	0.02	160	1.65	1.7	0.02	46	0.74	58	2.07	NS		NS	
	29-Aug-00	291	4.6	0.03	1.1	0.01	74	0.76	1.6	0.02	21	0.34	61	2.17	NS		NS	
	25-Sep-00	318	8.1	0.05	1.1	0.01	23	0.24	1.4	0.01	12	0.19	86	3.07	NS		NS	
	23-Aug-01	650	5.0	0.03	<2.5	-	190	1.96	NA	-	44	0.70	<10	-	NS		NS	
ave of duplicates[(1)]	11-Oct-01	699	2.9	0.02	1.2	0.01	150	1.55	NA	-	36	0.58	<10	-	NS		NS	
	7-Nov-01	726	<1.0	-	<1.0	-	3.2	0.03	<1.0	-	9.9	0.16	37	1.32	NS		NS	
	28-Nov-01	747	<1	-	<1	-	8.7	0.09	<1	-	18	0.29	43	1.53	NS		NS	
	18-Dec-01	767	<1.0	-	<1.0	-	5.5	0.06	<1.0	-	8.3	0.13	26	0.93	NS		NS	
	25-Apr-02	894	<1.0	-	<1.0	-	8.1	0.08	<1.0	-	14	0.22	24	0.86	<10	-	10,000	623.32
B3 (MW140)	12-Nov-99	0	880	5.31	11	0.08	180	1.86	<10	-	<30	-	<2	-	NS		NS	
	15-Feb-00	95	730	4.40	15	0.11	170	1.75	<12	-	<30	-	<2	-	NS		NS	
	16-Mar-00	125	710	4.28	22	0.17	190	1.96	<10	-	<10	-	<2	-	NS		NS	
	3-May-00	173	29	0.17	2	0.02	610	6.29	< 1	-	< 1	-	<2	-	NS		NS	
	22-May-00	192	62	0.37	100	0.76	410	4.23	1.3	0.01	2.3	0.04	<2	-	NS		NS	
	5-Jun-00	206	39	0.24	160	1.22	340	3.51	1.6	0.02	<2.0	-	<2	-	NS		NS	
	27-Jun-00	228	18	0.11	21	0.16	460	4.75	2.2	0.02	3.6	0.06	<2	-	NS		NS	
ave of duplicates	17-Jul-00	248	8.55	0.05	2.95	0.02	440	4.54	2.15	0.02	37.5	0.60	<2	-	NS		NS	
ave of duplicates	7-Aug-00	269	7.6	0.05	1.6	0.01	150	1.55	2.15	0.02	59	0.94	82	2.92	NS		NS	
	29-Aug-00	291	5.5	0.03	1	0.01	55	0.57	1.7	0.02	27	0.43	107	3.81	NS		NS	
	25-Sep-00	318	9.5	0.06	-	-	12	0.12	1.7	0.02	12	0.19	110	3.92	NS		NS	
	23-Aug-01	650	120	0.72	10	0.08	45	0.46	NA	-	2.0	0.03	<10	-	NS		NS	
	11-Oct-01	699	<2.0	-	<2.0	-	160	1.65	NA	-	41.0	0.66	<10	-	NS		NS	
	7-Nov-01	726	<1.0	-	<1.0	-	5.0	0.05	<1.0	-	8.9	0.14	45.5	1.62	NS		NS	
ave of duplicates	28-Nov-01	747	1.5	0.01	<1	-	16.5	0.17	<1	-	17.0	0.27	55	1.96	NS		NS	
	19-Dec-01	768	<1.0	-	<1.0	-	16	0.17	<1.0	-	18.0	0.29	31	1.11	NS		NS	
	19-Mar-02	857	<1.0	-	<1.0	-	2.6	0.03	<1.0	-	7.8	0.12	NS	-	NS		NS	
	25-Apr-02	894	<1.0	-	<1.0	-	14.0	0.14	<1.0	-	12.0	0.19	15	0.53	<10	-	7,500	467.49

KELLY AFB BIOAUGMENTATION PILOT TEST (Continued)

Well	Date	Day	PCE		TCE		*cis*-12DCE		*trans*-12DCE		VC		Ethene		Ethane			
E1 (MW142)	12-Nov-99	0	1080	6.51	20	0.15	200	2.06	<10	-	<30	-	<2	-	NS		NS	
ave of duplicates	15-Feb-00	95	695	4.19	0	-	195	2.01	0	-	0	-	<2	-	NS		NS	
ave of duplicates	16-Mar-00	125	710	4.28	22	0.17	180	1.86	0	-	0	-	<2	-	NS		NS	
	3-May-00	173	230	1.39	11	0.08	370	3.82	< 1	-	< 1	-	<2	-	NS		NS	
	22-May-00	192	240	1.45	67	0.51	300	3.09	1.5	0.02	<2	-	<2	-	NS		NS	
	5-Jun-00	206	290	1.75	120	0.91	280	2.89	1.3	0.01	<2	-	<2	-	NS		NS	
	27-Jun-00	228	220	1.33	27	0.21	350	3.61	1.9	0.02	<3	-	<2	-	NS		NS	
	17-Jul-00	248	170	1.03	8.3	0.06	370	3.82	1.8	0.02	20	0.32	<2	-	NS		NS	
	7-Aug-00	269	180	1.09	7	0.05	150	1.55	1.7	0.02	61	0.98	45	1.60	NS		NS	
	29-Aug-00	291	170	1.03	5.9	0.04	92	0.95	1.7	0.02	15	0.24	52	1.85	NS		NS	
	25-Sep-00	318	130	0.78	4	0.03	53	0.55	1.3	0.01	-	-	72	2.57	NS		NS	
	19-Dec-01	768	94	0.57	4.2	0.03	61	0.63	<3.0	-	5.6	-	11	0.39	NS		NS	
	25-Apr-02	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		NS	
E2 (MW141)	12-Nov-99	0	1120	6.75	21	0.16	220	2.27	<10	-	<30	-	<2	-	NS		NS	
	15-Feb-00	95	660	3.98	<12	-	160	1.65	<12	-	<30	-	<2	-	NS		NS	
	16-Mar-00	125	850	5.13	24	0.18	200	2.06	<10	-	<10	-	<2	-	NS		NS	
	3-May-00	173	580	3.50	14	0.11	160	1.65	< 1	-	< 1	-	<2	-	NS		NS	
	22-May-00	192	500	3.02	12	0.09	160	1.65	1.1	0.01	<2	-	<2	-	NS		NS	
	5-Jun-00	206	450	2.71	21	0.16	160	1.65	1.1	0.01	<2	-	<2	-	NS		NS	
	27-Jun-00	228	390	2.35	22	0.17	210	2.17	<1	-	<3	-	<2	-	NS		NS	
	17-Jul-00	248	440	2.65	15	0.11	260	2.68	1.1	0.01	5.3	0.08	<2	-	NS		NS	
	7-Aug-00	269	420	2.53	11.2	0.09	240	2.48	<1	-	15	0.24	4.4	0.16	NS		NS	
	29-Aug-00	291	310	1.87	7.3	0.06	140	1.44	<1	-	12	0.19	12	0.43	NS		NS	
	25-Sep-00	318	250	1.51	5.2	0.04	100	1.03	1.1	0.01	8.3	0.13	35	1.25	NS		NS	
	23-Aug-01	650	54	0.33	1.9	0.01	14	0.14	NA	-	<1.0	-	<10	-	NS		NS	
	11-Oct-01	699	180	1.09	8.8	0.07	95	0.98	NA	-	5.1	0.08	<10	-	NS		NS	
	7-Nov-01	726	460	2.77	15	0.11	180	1.86	<3.0	-	6.6	0.11	<10	-	NS		NS	
	28-Nov-01	747	130	0.78	6	0.05	84	0.87	<2	-	3.2	0.05	<10	-	NS		NS	
	19-Dec-01	768	80	0.48	3.1	0.02	40	0.41	<2.0	-	<2.0	-	<10		NS		NS	
	25-Apr-02	895	32	0.19	6.6	0.05	160	1.65	<2.0		8.8	0.14	<10		<10	-	5,900	367.76

KELLY AFB BIOAUGMENTATION PILOT TEST (Continued)

Well	Date	Day	PCE		TCE		*cis*-12DCE		*trans*-12DCE		VC		Ethene		Ethane			
E3 (MW139)	12-Nov-99	0	1110	6.69	17	0.13	230	2.37	<10	-	<30	-	<2	-				
T1 (MW138)	12-Nov-99	0	1040	6.27	20	0.15	220	2.27	<10	-	<30	-	<2	-	NS		NS	
	15-Feb-00	95	650	3.92	<12	-	120	1.24	<12	-	<30	-	<2	-	NS		NS	
	16-Mar-00	125	790	4.76	24	0.18	190	1.96	<10	-	<10	-	<2	-	NS		NS	
	3-May-00	173	33	0.20	7.5	0.06	610	6.29	< 1	-	< 1	-	<2	-	NS		NS	
	22-May-00	192	87	0.52	96	0.73	420	4.33	1.4	0.01	2.4	0.04	<2	-	NS		NS	
	5-Jun-00	206	57	0.34	130	0.99	310	3.20	1.6	0.02	<2.0	-	<2	-	NS		NS	
	27-Jun-00	228	26	0.16	13	0.10	460	4.75	2.1	0.02	4.2	0.07	<2	-	NS		NS	
	17-Jul-00	248	6.2	0.04	2.3	0.02	480	4.95	2.2	0.02	28	0.45	<2	-	NS		NS	
	7-Aug-00	269	5.4	0.03	3	0.02	84	0.87	1.9	0.02	41	0.66	110	3.92	NS		NS	
	29-Aug-00	291	3.9	0.02	1.5	0.01	49	0.51	1.6	0.02	24	0.38	98	3.49	NS		NS	
	25-Sep-00	318	2.8	0.02	1.1	0.01	23	0.24	1.4	0.01	11	0.18	120	4.28	NS		NS	
	23-Aug-01	650	18	0.11	8.2	0.06	190	1.96	NA	-	44	0.70	<10	-	NS		NS	
	11-Oct-01	699	<2.0	-	<2.0	-	160	1.65	NA	-	31	0.50	<10	-	NS		NS	
	7-Nov-01	726	<1.0	-	<1.0	-	9.6	0.10	<1.0	-	23	0.37	30	1.07	NS		NS	
	28-Nov-01	747	<1	-	<1	-	3.5	0.04	<1	-	8.9	0.14	48	1.71	NS		NS	
	19-Dec-01	768	<1.0	-	<1.0	-	3.1	0.03	<1.0	-	7.1	0.11	46	1.64	NS		NS	
	25-Apr-02	895	<1.0	-	<1.0	-	12	0.12	<1.0	-	13	0.21	13	0.46	<10	-	7,200	448.79
T2 (MW135)	12-Nov-99	0	970	5.85	18	0.14	220	2.27	<10	-	<30	-	<2	-	NS		NS	
	3-May-00	173	49	0.30	3.1	0.02	470	4.85	< 1	-	< 1	-	<2	-	NS		NS	
ave of duplicates	22-May-00	192	135	0.81	98	0.75	370	3.82	1.2	0.01	3	0.05	<2	-	NS		NS	
	5-Jun-00	206	79	0.48	170	1.29	350	3.61	1.9	0.02	2.3	0.04	<2	-	NS		NS	
	27-Jun-00	228	30	0.18	22	0.17	500	5.16	2.4	0.02	9.6	0.15	<2	-	NS		NS	
	17-Jul-00	248	9.7	0.06	4.5	0.03	500	5.16	2.4	0.02	21	0.34	<2	-	NS		NS	
	7-Aug-00	269	6.4	0.04	2.6	0.02	240	2.48	2	0.02	75	1.20	48	1.71	NS		NS	
	29-Aug-00	291	5.4	0.03	3.2	0.02	110	1.13	1.7	0.02	40	0.64	86	3.07	NS		NS	
	25-Sep-00	318	10	0.06	2.1	0.02	39	0.40	1.6	0.02	10	0.16	110	3.92	NS		NS	
	19-Dec-01	768	<1.0	-	<1.0	-	11	0.11	<1.0	-	10	0.16	38	1.35	NS		NS	

KELLY AFB BIOAUGMENTATION PILOT TEST (Continued)

Well	Date	Day	PCE		TCE		*cis*-12DCE		*trans*-12DCE		VC		Ethene		Ethane			
	25-Apr-02	895	<1.0	-	<1.0	-	14	0.14	<1.0	-	13	0.21	16	0.57	<10	-	6,500	405.16
I1 (RW162)	22-Aug-01	-	<2.5	-	<2.5	-	190	1.96	NA	-	73	1.17	<10	-	NS		NS	
	12-Oct-01	-	100	-	5.4	-	120	1.24	NA	-	17	0.27	<10	-	NS		NS	
	6-Nov-01	725	350	2.11	10	0.08	110	1.14	<2.0	-	6.6	0.11	<10	-	NS		NS	
	29-Nov-01	748	260	1.57	11	0.08	120	1.24	<4	-	6.4	0.10	11	0.39	NS		NS	
	18-Dec-01	767	100	0.60	4.6	0.04	52	0.54	<2.0	-	3.8	0.06	<10	-	NS		NS	
	19-Mar-02	857	82	0.49	10	0.08	140	1.44	<1.0	-	4.8	0.08	NS	-	NS		NS	
	26-Apr-02	894	37	0.22	6.4	0.05	110	1.14	<1.0	-	11	0.18	<10	-	<10	-	9,400	585.93

- = not calculated

Plot 1 was bioaugemented on May 6, 2000, 176 days from the start of the system.

(1) TCE concentrations were not averaged for Oct. 11, 2001 B2sample because the reporting limit was changed from 1.0 µg/L to 2.0 µg/L, resulting in an ND measurement for the duplicate.

March 2002 Sampling limited to 3 wells

August 2001 Sampling Event

VOCs		VC	cis-12DCE	TCE	PCE	Methane	Ethane	Ethene
Well	Date	µg/L	µg/L	µg/L	µg/L	µg/mL	µg/mL	µg/mL
Bioaugmentation Plot								
B1/MW136	8/23/2001	8.4	4.5	<1.0	<1.0	5000	23	22
B2/MW137	8/23/2001	44	190	<2.5	5	2100	<10	<10
T1/MW138	8/23/2001	44	190	8.2	18	890	<10	<10
B3/MW140	8/23/2001	2.0	45	10	120	64	<10	<10
E2/MW-141	8/23/2001	<1.0	14	1.9	54	<5	<10	<10
I1/RW162	8/23/2001	73	190	<2.5	<2.5	6900	<100	22
Oleate (Control) Plot								
I2/MW202	8/22/2001	<1.0	48	11	79	210	<10	<10
B6/MW203	8/22/2001	<1.0	28	3.3	98	<5	<10	<10
B5/MW204	8/22/2001	<1.0	31	3.2	94	6	<10	<10
B4/MW205	8/22/2001	<1.0	29	2.9	86	<5	<10	<10
E5/MW-208	8/22/2001	<1.0	28	3.7	110	<5	<10	<10

VFAs		Lactate	Acetate	Propionate	Butyrate
Well	Date	mg/L	mg/L	mg/L	mg/L
Bioaugmentation Plot					
B1/MW136	8/23/2001	<0.1	<0.09	<0.08	<0.2
B2/MW137	8/23/2001	<0.1	<0.09	<0.08	<0.2
T1/MW138	8/23/2001	<0.1	<0.09	<0.08	<0.2
B3/MW140	8/23/2001	<0.1	<0.09	<008	<0.2
E2/MW-141	8/23/2001	<0.1	<0.09	<0.08	<0.2
I1/RW162	8/23/2001	<0.1	<0.09	<0.08	<0.5
Oleate (Control) Plot					
I2/MW202	8/22/2001	<0.1	<0.09	<0.08	<0.2
B6/MW203	8/22/2001	<0.1	<0.09	<0.08	<0.2

August 2001 Sampling Event (Continued)

B5/MW204	8/22/2001	<0.1	<0.09	<0.08	<0.2
B4/MW205	8/22/2001	<0.1	<0.09	<0.08	<0.2
E5/MW-208	8/22/2001	<0.1	<0.09	<0.08	<0.2

Inorganics		**Nitrate**	**Nitrite**	**Sulfate**	**Bromide**
Well	**Date**	mg/L	mg/L	mg/L	mg/L
Bioaugmentation Plot					
B1/MW136	8/23/2001	<0.5	<0.5	9.5	1.1
B2/MW137	8/23/2001	<0.5	<0.5	16	0.88
T1/MW138	8/23/2001	<0.5	<0.5	13	0.4
B3/MW140	8/23/2001	<0.5	<0.5	13	0.69
E2/MW-141	8/23/2001	0.72	<0.5	15	0.67
I1/RW162	8/23/2001	<0.5	<0.5	7.4	1.1
Oleate (Control) Plot					
I2/MW202	8/22/2001	0.75	<0.5	26	1.3
B6/MW203	8/22/2001	1.3	<0.5	25	1
B5/MW204	8/22/2001	1.3	<0.5	28	1.4
B4/MW205	8/22/2001	1	<0.5	22	1.5
E5/MW-208	8/22/2001	1.3	<0.5	47	1.1

D. ethenogenes			
Well	**Date**	**Present**	**Not Present**
Bioaugmentation Plot			
B2/MW137	8/24/2001	+	
E2/MW-141	8/24/2001	+	
I1/RW162	8/24/2001	++	
Oleate (Control) Plot			
B6/MW203	8/24/2001		X
B4/MW205	8/24/2001		X

October 2001 Sampling Event

VOCs		VC	cis-12DCE	TCE	PCE	Methane	Ethane	Ethene
Well	Date	µg/L	µg/L	µg/L	µg/L	µg/mL	µg/mL	µg/mL
Bioaugmentation Plot								
B1/MW136	10/11/2001	23	150	2.9	5.0	1400	<10	<10
B2/MW137	10/11/2001	37	160	1.2	2.8	1000	<10	<10
B2/MW137	10/11/2001	35	140	<2.0	3	2200	<10	<10
T1/MW138	10/11/2001	31	160	<2.0	<2.0	2200	<10	<10
B3/MW140	10/11/2001	41	160	<2.0	<2.0	250	<10	<10
E2/MW-141	10/11/2001	5.1	95	8.8	180	2100	<10	<10
I1/RW162	10/12/2001	17	120	5.4	100	1400	<100	<100
Oleate (Control) Plot								
I2/MW202	10/11/2001	<1.0	31	4.1	91	<5	<10	<10
B6/MW203	10/11/2001	<1.0	27	3.6	77	<5	<10	<10
B5/MW204	10/11/2001	<1.0	27	3.7	80	<5	<10	<10
B4/MW205	10/11/2001	<1.0	26	3.7	77	<5	<10	<10
E5/MW-208	10/11/2001	<1.0	23	3.4	71	<5	<100	<10

VFAs		Lactate	Acetate	Propionate	Butyrate
Well	Date	mg/L	mg/L	mg/L	mg/L
Bioaugmentation Plot					
B1/MW136	10/11/2001	<0.1	<0.09	<0.08	<0.2
B2/MW137	10/11/2001	<0.1	<0.09	<0.08	<0.2
B2/MW137	10/12/2001	<0.1	<0.09	<0.08	<0.2
T1/MW138	10/11/2001	<0.1	<0.09	<0.08	<0.2
B3/MW140	10/11/2001	<0.1	<0.09	<0.08	<0.2
E2/MW-141	10/11/2001	<0.1	<0.09	<0.08	<0.2
I1/RW162	10/12/2001	<0.1	<0.09	<0.08	<0.2

Oleate (Control) Plot					
I2/MW202	10/11/2001	<0.1	<0.09	<0.08	<0.2
B6/MW203	10/11/2001	<0.1	<0.09	<0.08	<0.2
B5/MW204	10/11/2001	<0.1	<0.09	<0.08	<0.2
B4/MW205	10/11/2001	<0.1	<0.09	<0.08	<0.2
E5/MW-208	10/11/2001	<0.1	<0.09	<0.08	<0.2

Inorganics		**Nitrate**	**Nitrite**	**Sulfate**	**Bromide**
Well	**Date**	mg/L	mg/L	mg/L	mg/L
Bioaugmentation Plot					
B1/MW136	10/11/2001	<1.0	<1.0	21	0.47
B2/MW137	10/11/2001	<1.0	<1.0	16	0.55
B2/MW137	10/11/2001	<1.0	<1.0	17	0.56
T1/MW138	10/11/2001	<1.0	<1.0	17	0.52
B3/MW140	10/11/2001	<1.0	<1.0	15	0.50
E2/MW-141	10/11/2001	<1.0	<1.0	13	0.31
I1/RW162	10/12/2001	<1.0	<1.0	16	0.49
Oleate (Control) Plot					
I2/MW202	10/11/2001	1.8	<1.0	24	0.82
B6/MW203	10/11/2001	1.7	<1.0	30	0.84
B5/MW204	10/11/2001	1.8	<1.0	25	0.83
B4/MW205	10/11/2001	1.8	<1.0	31	0.86
E5/MW-208	10/11/2001	1.5	<1.0	22	0.77

November 2001 First Sampling Event

VOCs		VC	cis-12DCE	TCE	PCE	Methane	Ethane	Ethene
Well	Date	µg/L	µg/L	µg/L	µg/L	µg/mL	µg/mL	µg/mL
Bioaugmentation Plot								
B1/MW136	11/7/2001	9.9	5.4	<1.0	<1.0	9600.0	<10	38
B2/MW137	11/7/2001	9.9	3.2	<1.0	<1.0	7800	<10	<10
T1/MW138	11/7/2001	23	9.6	<1.0	<1.0	6800	<10	<10
B3/MW140	11/7/2001	8.9	5.0	<1.0	<1.0	8950	<10	45.5
E2/MW-141	11/7/2001	6.6	180	15	460	1400	<10	<10
I1/RW162	11/6/2001	6.6	110	10	350	2600	<100	<10
Oleate (Control) Plot								
I2/MW202	11/6/2001	<1.0	50	2.4	51	1800	<10	<10
B6/MW203	11/6/2001	<1.0	88	1.7	6.6	3800	<10	<10
B5/MW204	11/6/2001	<1.0	74	2.9	11	4600	<10	<10
B4/MW205	11/6/2001	<1.0	77	2.2	12	4400	<10	<10
E5/MW-208	11/6/2001	<1.0	51	1.8	32	2000.0	<100	<10

VFAs		Lactate	Acetate	Propionate	Butyrate
Well	Date	mg/L	mg/L	mg/L	mg/L
Bioaugmentation Plot					
B1/MW136	11/7/2001	<0.1	0.78	<0.08	<0.2
B2/MW137	11/7/2001	<0.1	6.91	<0.08	<0.2
T1/MW138	11/7/2001	<0.1	2.77	<0.08	<0.2
B3/MW140	11/7/2001	<0.1	5.37	<0.08	<0.2
E2/MW-141	11/7/2001	<0.1	<0.09	<0.08	<0.2
I1/RW162	11/6/2001	<0.1	93.9	<0.08	<0.2
Oleate (Control) Plot					
I2/MW202	11/6/2001	<0.1	<0.09	<0.08	<0.2
B6/MW203	11/6/2001	<0.1	5.46	<0.08	<0.2

November 2001 First Sampling Event (Continued)

B5/MW204	11/6/2001	<0.1	6.96	<0.08	<0.2
B4/MW205	11/6/2001	<0.1	11.1	<0.08	<0.2
E5/MW-208	11/6/2001	<0.1	<0.09	<0.08	<0.2

Inorganics		**Nitrate**	**Nitrite**	**Sulfate**	**Bromide**
Well	**Date**	mg/L	mg/L	mg/L	mg/L
Bioaugmentation Plot					
B1/MW136	11/7/2001	<0.25	<0.25	1.9	0.55
B2/MW137	11/7/2001	<0.25	<0.25	1.5	0.55
T1/MW138	11/7/2001	<0.25	<0.25	1.8	0.72
B3/MW140	11/7/2001	<0.25	<0.25	2.5	0.52
E2/MW-141	11/7/2001	0.83	<0.25	12	0.41
I1/RW162	11/6/2001	0.27	<0.25	12	0.52
Oleate (Control) Plot					
I2/MW202	11/6/2001	0.85	<0.25	25	0.8
B6/MW203	11/6/2001	<0.25	<0.25	20	0.84
B5/MW204	11/6/2001	<0.25	<0.25	22	0.83
B4/MW205	11/6/2001	<0.25	<0.25	19	0.82
E5/MW-208	11/6/2001	0.79	<0.25	24	0.77

November 2001 Second Sampling Event

VOCs		VC	cis-12DCE	TCE	PCE	Methane	Ethane	Ethene
Well	Date	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Bioaugmentation Plot								
B1/MW136	11/28/2001	32	29	<1.0	<1.0	13000	15	62
B2/MW137	11/28/2001	18	8.7	<1.0	<1.0	11000	15	43
T1/MW138	11/28/2001	8.9	3.5	<1.0	<1.0	9400	12	48
B3/MW140	11/28/2001	17	16.5	<1.0	1.5	11000	<10	55
E2/MW-141	11/28/2001	3.2	84	6.0	130	480	<10	<10
I1/RW162	11/29/2001	6.4	120	11	260	2400	<10	11
Oleate (Control) Plot								
I2/MW202	11/29/2001	<1	54	2.4	43	3600	<10	<10
B6/MW203	11/29/2001	<2.5	80	<2.5	<2.5	8600	<10	<10
B5/MW204	11/29/2001	<1	73	<1	2.2	7900	<10	<10
B4/MW205	11/29/2001	<1	67	1.4	6.7	6500	<10	<10
E5/MW-208	11/29/2001	<1	69	1.4	18	5100	<10	<10

VFAs		Lactate	Acetate	Propionate	Butyrate
Well	Date	mg/L	mg/L	mg/L	mg/L
Bioaugmentation Plot					
B1/MW136	11/28/2001	<0.1	<0.09	<0.08	<0.2
B2/MW137	11/28/2001	0.4	<0.09	<0.08	<0.2
T1/MW138	11/28/2001	0.5	<0.09	<0.08	<0.2
B3/MW140	11/28/2001	0.3	<0.09	<0.08	<0.2
E2/MW-141	11/28/2001	0.1	<0.09	<0.08	<0.2
I1/RW162	11/29/2001	0.1	<0.09	<0.08	<0.2
Oleate (Control) Plot					
I2/MW202	11/29/2001	<0.1	<0.09	<0.08	<0.2
B6/MW203	11/29/2001	<0.1	<0.09	<0.08	<0.2

Average of duplicates

November 2001 Second Sampling Event (Continued)

B5/MW204	11/29/2001	0.2	5.8	<0.08	<0.2
B4/MW205	11/29/2001	0.1	3.1	<0.08	<0.2
E5/MW-208	11/29/2001	<0.1	<0.09	<0.08	<0.2

Inorganics		**Nitrate**	**Nitrite**	**Sulfate**	**Bromide**	
Well	**Date**	mg/L	mg/L	mg/L	mg/L	
Bioaugmentation Plot						
B1/MW136	11/28/2001	<0.25	<0.25	4.0	0.55	
B2/MW137	11/28/2001	<0.25	<0.25	4.1	0.47	
T1/MW138	11/28/2001	<0.25	<0.25	2.4	0.48	
B3/MW140	11/28/2001	<0.25	<0.25	2.45	0.465	Average of duplicate
E2/MW-141	11/28/2001	0.45	<0.25	12	0.26	
I1/RW162	11/29/2001	0.66	<0.25	12	0.37	
Oleate (Control) Plot						
I2/MW202	11/29/2001	0.71	<0.25	21	0.72	
B6/MW203	11/29/2001	<0.25	<0.25	6.6	0.74	
B5/MW204	11/29/2001	<0.25	<0.25	9.5	0.71	
B4/MW205	11/29/2001	<0.25	<0.25	14	0.71	
E5/MW-208	11/29/2001	0.29	<0.25	17	0.76	

Note: The Field Blank recorded a sulfate concentration of 0.46 mg/L. All other anions were ND.

December 2001 Sampling Event

VOCs		VC	cis-12DCE	TCE	PCE	Methane	Ethane	Ethene
Well	Date	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Bioaugmentation Plot								
T2/MW135	12/18/2001	10	11	<1.0	<1.0	8700	10	38
B1/MW136	12/18/2001	8.3	8.8	<1.0	<1.0	11000	17	25
B2/MW137	12/18/2001	8.3	5.5	<1.0	<1.0	12000	22	26
T1/MW138	12/19/2001	7.1	3.1	<1.0	<1.0	12000	11	46
B3/MW140	12/19/2001	18	16.0	<1.0	<1.0	7400	<10	31
E2/MW-141	12/19/2001	<2.0	40	3.1	80	170	<10	<10
E1/MW-142	12/19/2001	5.6	61	4.2	94	2900	<10	11
I1/RW162	12/18/2001	3.8	52	4.6	100	2900	<10	<10
Oleate (Control) Plot								
I2/MW202	12/18/2001	<1.0	35	1.4	25	3800	<10	<10
B6/MW203	12/17/2001	<2.0	56	<2.0	<2.0	13000	<10	<10
B5/MW204	12/18/2001	1.6	55	<1.0	<1.0	12000	<10	<10
B4/MW205	12/18/2001	2.1	59	<1.0	<1.0	10000	<10	<10
E5/MW-208	12/17/2001	<1.0	41	1.1	17	5400	<10	<10

VFAs		Lactate	Acetate	Propionate	Butyrate
Well	Date	mg/L	mg/L	mg/L	mg/L
Bioaugmentation Plot					
T2/MW135	12/18/2001	NS	<0.09	<0.08	<0.2
B1/MW136	12/18/2001	NS	0.13	<0.08	<0.2
B2/MW137	12/18/2001	NS	0.10	<0.08	<0.2
T1/MW138	12/19/2001	NS	<0.09	<0.08	<0.2
B3/MW140	12/19/2001	NS	<0.09	<0.08	<0.2
E2/MW-141	12/19/2001	NS	<0.09	<0.08	<0.2
E1/MW-142	12/18/2001	NS	<0.09	<0.08	<0.2
I1/RW162	12/18/2001	NS	<0.09	<0.08	<0.2

December 2001 Sampling Event (Continued)

Oleate (Control) Plot					
I2/MW202	12/18/2001	NS	<0.09	<0.08	<0.2
B6/MW203	12/17/2001	NS	1.1	<0.08	<0.2
B5/MW204	12/18/2001	NS	0.85	<0.08	<0.2
B4/MW205	12/18/2001	NS	18.7	<0.08	<0.2
E5/MW-208	12/17/2001	NS	<0.09	<0.08	<0.2

Inorganics		Nitrate	Nitrite	Sulfate	Bromide
Well	**Date**	mg/L	mg/L	mg/L	mg/L
Bioaugmentation Plot					
T2/MW135	12/18/2001	<0.25	<0.25	8.0	0.40
B1/MW136	12/18/2001	<0.25	<0.25	1.6	0.34
B2/MW137	12/18/2001	<0.25	<0.25	3.4	0.40
T1/MW138	12/19/2001	<0.25	<0.25	4.2	0.47
B3/MW140	12/19/2001	<0.25	<0.25	6.1	0.41
E2/MW-141	12/19/2001	0.45	<0.25	13	<0.25
E1/MW-142	12/19/2001	0.56	<0.25	14	0.46
I1/RW162	12/18/2001	0.29	<0.25	13	0.35
Oleate (Control) Plot					
I2/MW202	12/18/2001	<0.25	<0.25	26	0.63
B6/MW203	12/17/2001	<0.25	<0.25	1.1	0.66
B5/MW204	12/18/2001	<0.25	<0.25	10	0.65
B4/MW205	12/18/2001	<0.25	<0.25	6.1	0.70
E5/MW-208	12/17/2001	0.27	<0.25	16	0.67

NS = not sampled

March 2002 Sampling Event

VOCs		VC	cis-12DCE	trans-1,2DCE	TCE	PCE
Well	**Date**	µg/L	µg/L	µg/L	µg/L	µg/L
Bioaugmentation Plot						
B1/MW136	3/19/2002	16	14	<1.0	<1.0	<1.0
B3/MW140	3/19/2002	7.8	2.6	<1.0	<1.0	<1.0
I1/RW162	3/19/2002	4.8	140	<1.0	10	82

VFAs		Lactate	Acetate	Propionate	Butyrate
Well	**Date**	mg/L	mg/L	mg/L	mg/L
Bioaugmentation Plot					
B1/MW136	3/19/2002	<0.1	0.91	<0.08	<0.2
B3/MW140	3/19/2002	0.7	<0.09	<0.08	<0.2
I1/RW162	3/19/2002	<0.1	21.9	<0.2	<0.2

Well	Date	Sulfate (mg/L)
B1/MW136	3/19/2002	23
B3/MW140	3/19/2002	8.6
I1/RW162	3/19/2002	78

April 2002 Sampling Event

VOCs		VC	cis-12DCE	TCE	PCE	Methane	Ethane	Ethene	trans-1,2DCE
Well	Date	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Bioaugmentation Plot									
T2/MW135	4/25/2002	13	14	<1.0	<1.0	6,500	<10	16	<1.0
B1/MW136	4/25/2002	7.7	4.0	<1.0	<1.0	9,700	<10	18	<1.0
B2/MW137	4/25/2002	14	8.1	<1.0	<1.0	10,000	<10	24	<1.0
T1/MW138	4/25/2002	13	12	<1.0	<1.0	7,200	<10	13	<1.0
B3/MW140	4/25/2002	12	14.0	<1.0	<1.0	7,500	<10	15	<1.0
E2/MW-141	4/25/2002	8.8	160	6.6	32	5,900	<10	<10	<2.0 V
E1/MW-142	4/25/2002	NS	NS	NS	NS	NS	NS	NS	NS
I1/RW162	4/26/2002	11	110	6.4	37	9,400	<10	<10	<1.0
Oleate (Control) Plot									
I2/MW202	4/25/2002	1.3	11	2.4	36	4,100	<10	<10	<1.0
B6/MW203	4/25/2002	<1.0	<1.0	<1.0	<1.0	10,000	<10	<10	<1.0
B5/MW204	4/25/2002	<1.0	<1.0	<1.0	<1.0	2,000	<10	<10	<1.0
B4/MW205	4/25/2002	2.0	3.7	<1.0	<1.0	11,000	<10	<10	<1.0
E5/MW-208	4/24/2002	<1.0	8.1	2.0	34	4200	<10	<10	<1.0

VFAs		Lactate	Acetate	Propionate	Butyrate
Well	Date	mg/L	mg/L	mg/L	mg/L
Bioaugmentation Plot					
T2/MW135	4/25/2002	<0.2	<0.2	<0.08	<0.2
B1/MW136	4/25/2002	<0.2	<0.2	<0.08	<0.2
B2/MW137	4/25/2002	<0.2	<0.2	<0.08	<0.2
T1/MW138	4/25/2002	<0.2	<0.2	<0.08	<0.2
B3/MW140	4/25/2002	<0.2	<0.2	<0.08	<0.2
E2/MW-141	4/25/2002	<0.2	<0.2	<0.08	<0.2
E1/MW-142	4/25/2002	NS	NS	NS	NS
I1/RW162	4/26/2002	<4.0	249	<2.0	<0.2

April 2002 Sampling Event (Continued)

Oleate (Control) Plot					
I2/MW202	4/25/2002	<0.2	3.4	<0.08	<0.2
B6/MW203	4/25/2002	0.3	7.1	0.7	<0.2
B5/MW204	4/25/2002	2.3	140	9.7	1.5
B4/MW205	4/25/2002	<1.0	102	6.9	0.7
E5/MW-208	4/24/2002	<0.2	2.6	0.7	<0.2

Inorganics		Nitrate	Nitrite	Sulfate	Sulfide	Bromide	Iron	Ferrous Iron
Well	**Date**	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Bioaugmentation Plot								
T2/MW135	4/25/2002	<0.25 *	<0.25 *	15.0	<0.1	0.63	1.4	1.0
B1/MW136	4/25/2002	<0.25	<0.25	14.0	<0.1	0.52	3.6	3.0
B2/MW137	4/25/2002	<0.25 *	<0.25 *	16	<0.1	0.60	3.0	2.9
T1/MW138	4/25/2002	<0.25 *	<0.25 *	13	<0.1	0.58	2	1.9
B3/MW140	4/25/2002	<0.25 *	<0.25 *	13	<0.1	0.59	0.96	1.0
E2/MW-141	4/25/2002	<0.25 *	<0.25 *	13	0.1	0.60	1.1	0.9
E1/MW-142	4/25/2002	NS	NS	NS	NS	NS	NS	NS
I1/RW162	4/25/2002	<0.25 *	<0.25 *	640	1.8	0.82	0.08	<0.1
Oleate (Control) Plot								
I2/MW202	4/25/2002	0.46	<0.25 *	16	<0.1	0.48	<0.05	<0.1
B6/MW203	4/25/2002	<0.25 *	<0.25 *	1.4	0.1	0.53	2.1	2.1
B5/MW204	4/25/2002	<0.25 *	<0.25 *	0.33	0.2	0.62	11	11
B4/MW205	4/25/2002	<0.25 *	<0.25 *	0.68	0.9	0.58	7.9	7.5
E5/MW-208	4/24/2002	1.1 *	<0.25 *	17	<0.1	0.48	1.5	1.2

NS = not sampled

V = Reporting Limits were increased due to high concentrations of target analytes.

* The concentrations of Nitrite (NO2)-N and Nitrate (NO3)-N were determined using an acid preserved sub-sample. The value for the total sum of Nitrate-N plus Nitrite-N is considered to be accurate. The concentrations of the individual speciated parameters of Nitrite (NO2)-N and Nitrate (NO3)-N may not be accurate due to alterations of the relative proportions of the two compounds by the preservation process.

Field Measurements

AUGUST

Field Readings		pH	Temp	Cond.	DO	ORP	Salinity	Turbidity
Well	**Date**	units	°C	µS/cm	mg/L	mV	%	NTUs
Bioaugmentation Plot								
B1/MW136	8/23/2001	6.49	24.2	0.454	0.9	-93.4	0.01	999
B2/MW137	8/23/2001	6.61	23.9	0.427	0.62	-57.3	0.01	788
T1/MW138	8/23/2001	7.00	23.9	0.356	0.95	-61.8	0.01	451
B3/MW140	8/23/2001	7.11	24.2	0.296	0.58	-27.6	0.01	999
E2/MW-141	8/23/2001	7.09	23.9	0.306	1.04	197.2	0.01	120
I1/RW162	8/23/2001	6.50	25.3	0.458	0.52	-146.5	0.01	0
Oleate (Control) Plot								
I2/MW202	8/22/2001	6.15	25.3	0.598	0.93	NM	0.02	NM
B6/MW203	8/22/2001	6.48	25.4	0.586	0.61	229.2	0.2	999
B5/MW204	8/22/2001	6.55	25.5	0.598	0.91	195.9	0.02	999
B4/MW205	8/22/2001	6.47	25.9	0.583	1.68	NM	0.02	NM
E5/MW-208	8/22/2001	6.62	24.3	0.586	0.79	246.5	0.02	NM

NM -not measured

OCTOBER

Field Readings		pH	Temp	Cond	DO	ORP	Salinity	Turbidity
Well	**Date**	units	°C	µS/cm	mg/L	mV	%	NTUs
Bioaugmentation Plot								
B1/MW136	10/12/2001	NS	NS	NS	2.4	-115.7	NS	NS
B2/MW137	10/12/2001	NS	NS	NS	1.46	-82.8	NS	NS
T1/MW138	10/12/2001	NS	NS	NS	1.7	-109.3	NS	NS
T2	10/12/2001	NS	NS	NS	2.38	12.7 / 27.6	NS	NS
B3/MW140	10/12/2001	NS	NS	NS	1.77	-28 / -53.2	NS	NS
E2/MW-141	10/12/2001	NS	NS	NS	2	88.2	NS	NS
E1	10/12/2001	NS	NS	NS	2.4	80.6	NS	NS

Field Measurements (Continued)

I1/RW162	10/12/2001	NS	NS	NS	1.91	53.7	NS	NS
Oleate (Control) Plot								
I2/MW202	10/12/2001	NS	NS	NS	1.6	175.7	NS	NS
B6/MW203	10/12/2001	NS	NS	NS	1.98	212.2	NS	NS
B5/MW204	10/12/2001	NS	NS	NS	1.49	192.3	NS	NS
B4/MW205	10/12/2001	NS	NS	NS	2.16	192.4	NS	NS
T3		NS	NS	NS	1.94	218.3	NS	NS
T4		NS	NS	NS	1.95	232.2	NS	NS
E4		NS	NS	NS	1.62	171.9	NS	NS
E6		NS	NS	NS	1.77	157	NS	NS
E5/MW-208	10/12/2001	NS	NS	NS	1.2	171.9	NS	NS
NM -not measured								

NOVEMBER

1st Sampling Event

Field Readings		NS	**Temp**	**Cond**	**DO**	**ORP**	**Salinity**	**Turbidity**
Well	**Date**	units	°C	µS/cm	mg/L	mV	%	NTUs
Bioaugmentation Plot								
B1/MW136	11/7/2001	7.1	26.1	61	0.07	-108	0	0
B2/MW137	11/7/2001	7.18	25.2	61	2.46	-71	0	0
T1/MW138	11/7/2001	7.20	25.4	59	0.58	-87	0	0
B3/MW140	11/7/2001	7.26	24.2	58	0.11	-57	0	0
E2/MW-141	11/7/2001	7.32	25.5	50	0.59	11	0	0
I1/RW162	11/6/2001	7.33	25.7	63	3.5	-24	0	0
Oleate (Control) Plot								
I2/MW202	11/6/2001	7.23	27.7	66	0.1	-73	0	0
B6/MW203	11/6/2001	6.94	26.1	67	0.14	-33	0	110

Field Measurements (Continued)

B5/MW204	11/6/2001	7.20	27.4	66	1.44	-5	0	220
B4/MW205	11/6/2001	7.30	-100	67	0.59	-100	0	0
E5/MW-208	11/6/2001	6.8	26.0	67	0.37	70	0	190

NM -not measured

2nd Sampling Event

Field Readings		pH	Temp	Cond	DO	ORP	Salinity	Turbidity
Well	**Date**	units	°C	µS/cm	mg/L	mV	%	NTUs
Bioaugmentation Plot								
B1/MW136	11/28/2001	7.27	22.8	51	0.69	-100	0	310
B2/MW137	11/28/2001	7.29	22.9	55	0.87	-59	0	40
T1/MW138	11/28/2001	7.31	22.9	55	0.92	-29	0	20
B3/MW140	11/28/2001	6.89	22.7	58	0.52	16	0	550
E2/MW-141	11/28/2001	7.55	24.1	42	1.09	76	0	14
I1/RW162	11/29/2001	7.18	25.0	52	0.17	35	0	-10
Oleate (Control) Plot								
I2/MW202	11/29/2001	6.84	27.0	71	0.28	-82	0	240
B6/MW203	11/29/2001	6.93	24.9	71	0.35	-113	0	-2
B5/MW204	11/29/2001	6.92	26.5	73	0.19	-125	0	81
B4/MW205	11/29/2001	6.87	26.7	71	0.24	-116	0	-10
E5/MW-208	11/29/2001	6.88	27.8	71	0.65	48	0	0

NM -not measured

Field Measurements (Continued)

Dec-01

Field Readings		pH	Temp	Cond	DO	ORP	Salinity	Turbidity
Well	Date	units	°C	µS/cm	mg/L	mV	%	NTUs
Bioaugmentation Plot								
T2/MW135	12/18/2001	7.12	23.5	49	0.12	-177	0	88
B1/MW136	12/18/2001	7.12	24.7	47	0.10	-223	0	49
B2/MW137	12/18/2001	7.14	24.3	47	0.09	-160	0	20
T1/MW138	12/19/2001	6.65	24.0	58	3.0	-139	0	0
B3/MW140	12/19/2001	6.69	23.1	57	4.0	-126	0	41
E2/MW-141	12/19/2001	6.93	25.1	41	1.9	-154	0	87
E1/MW-142	12/19/2001	6.79	24.3	55	2.7	-137	0	0
I1/RW162	12/18/2001	7.31	26.2	40	0.03	-210	0	140
Oleate (Control) Plot								
I2/MW202	12/18/2001	7.16	27.4	55	0.06	-187	0	180
B6/MW203	12/17/2001	7.12	25.5	62	0	-139	0	20
B5/MW204	12/18/2001	6.67	26.9	58	NS	-209	0	4
B4/MW205	12/18/2001	7.08	27.2	59	NS	-196	0	110
E5/MW-208	12/17/2001	7.19	28.3	61	0	-35	0	53

03/19/2002 (3 wells sampled)

Field Readings		pH	Temp	Cond	DO	ORP	Salinity	Turbidity
Well	Date	units	°C	µS/cm	mg/L	mV	%	NTUs
Bioaugmentation Plot								
B1/MW136	3/19/2002	6.5	24.4	64	0.16	-171	0	0
B3/MW140	3/19/2002	6.57	23.1	60	0.4	-5	0	0
I1/RW162	3/19/2002	6.65	25.7	53	0.64	11	0	160

Field Measurements (Continued)

Apr-02

Field Readings		pH	Temp	Cond	DO	ORP	Salinity	Turbidity
Well	**Date**	units	°C	µS/cm	mg/L	mV	%	NTUs
Bioaugmentation Plot								
T2/MW135	4/25/2002	6.52	23.8	73	0.15	450	0.0	56.9
B1/MW136	4/25/2002	6.51	25.0	77	0.00	260	0.0	92.3
B2/MW137	4/25/2002	6.50	24.5	72	0.19	408	0.0	26.4
T1/MW138	4/25/2002	6.55	23.8	67	0.0	257	0.0	26.2
B3/MW140	4/25/2002	6.55	23.8	67	0.01	279	0.0	39.2
E2/MW-141	4/25/2002	6.60	23.1	61	0.13	294	0.0	-10.0
E1/MW-142	4/25/2002	NS	NS	NS	NS	NS	NS	NS
I1/RW162	4/26/2002	6.37	27.4	0.2	0.58	96	0.1	-10.0
Oleate (Control) Plot								
I2/MW202	4/25/2002	6.57	28.0	78	5.28	339	0.0	3.6
B6/MW203	4/25/2002	6.39	24.5	82	0.24	285	0.0	40.2
B5/MW204	4/25/2002	6.30	25.5	0.1	0.91	394	0.0	14.4
B4/MW205	4/25/2002	6.32	26.2	0.1	0.03	148	0.0	28.2
E5/MW-208	4/24/2002	6.49	24.4	69	0.0	410	0.0	50.4

Appendix C: Bioaugmentation White Paper

IN SITU BIOAUGMENTATION FOR GROUNDWATER REMEDIATION: STATE-OF-THE-PRACTICE AND RESEARCH NEEDS

A WHITE PAPER REVIEW

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1.0 INTRODUCTION

Bioaugmentation is the addition of a prepared culture of microorganisms with desired degradative properties to a contaminated medium to exploit the degradative qualities to enhance contaminant biotransformation. The cultures can be either pure or mixed microbial strains consisting of exogenous or indigenous microorganisms. Additionally, the cultures may contain genetically engineered microorganisms (GEMs) that have been altered to transform specific contaminants.

While the concept of bioaugmentation is not new, the historic aggressive promotion of various bacterial preparations that failed when applied in the field has hindered the acceptance of bioaugmentation as a viable technology for in situ applications. Early on, these attempts proved futile due to a lack of understanding of the specific requirements of the introduced cultures. For example, the competition between the indigenous and introduced microorganisms, limitations of microbial transport, transport of nutrient additions and electron-donor/acceptor substrates with the microbial culture, and the effects of the site geochemistry had not been investigated adequately at test sites. In addition, the results of some of the initial bioaugmentation field tests were ambiguous because those tests failed to employ experimental designs that provided sufficient control to allow a true test of the technology (e.g., it was difficult to separate the effect of introduced cultures from the effects of indigenous cultures, added nutrients, and dilution due to groundwater mixing).

The process of bioaugmentation has been applied successfully for many years by the wastewater treatment industry. For site remediation, recent scientific and engineering involvement has led to advancements in the understanding of the underlying principals of bioaugmentation. Field applications are now under way with success being achieved. Most of the successful applications have involved ex-situ treatment of contaminated soils that were excavated and placed into any number of reactor/treatment configurations. Success has been achieved using exogenous bacteria and fungi to treat contaminants ranging from the easily degraded petroleum hydrocarbons to the more recalcitrant PAHs and chlorinated aromatics and aliphatics. These successes are contributing to the advancement of the technology toward reliable and effective ex situ field applications. Unfortunately, there are vendors that promote the use of their products with little scientifically viable data to support their claims, and bioaugmentation is sometimes applied when a simpler biostimulation process would suffice. At the same time, biostimulation applications often fail because of a lack of proper recognition of the need for bioaugmentation (e.g., in the case of chlorinated ethenes).

The latest development in the bioaugmentation arena is the successful demonstration of the technology for in situ treatment of contaminated groundwater. Particularly in the case of chlorinated solvents, several rigorous demonstrations have been completed recently that establish the principal design parameters for successful implementation of the technology in sand and gravel aquifers. The success of bioaugmentation has benefited, in part, from an improved understanding of the key microorganisms that mediate biodegradation reactions (e.g., *Dehalococcoides ethenogenes* in reductive dechlorination of cis- dichloroethene and vinyl chloride). For certain bioaugmentation cultures, the development and application of DNA-fingerprinting techniques now allows practitioners to detect and monitor augmented cultures in the subsurface with relatively high sensitivity. Through application of these molecular monitoring techniques, researchers are now better able to directly measure and distinguish the effect of bioaugmentation from the background processes. The recent success of bioaugmentation has also been made possible by advancements in the understanding of factors affecting transport, interaction, and survival of exogenous cultures in contaminated groundwater environments.

As the popularity of monitored natural attenuation/bioremediation remedies has increased in recent years, the incidence of failure of these technologies has also increased. In some cases, these technologies have failed due to inadequate site characterization and/or poor engineering design. In many other cases, however, these technologies have failed because the rate of biodegradation achieved by indigenous microflora is insufficient to meet remediation objectives. This is fueling the trend towards the consideration and application of bioaugmentation to improve the rate and extent of biodegradation processes when the capabilities of the indigenous microbial population are insufficient. This trend is already occurring for sites contaminated with chlorinated solvents, methyl tert-butyl ether (MTBE), PAHs, and other recalcitrant contaminants. Sites contaminated with fuel hydrocarbons are more readily treated by intrinsic bioremediation and other technologies, and do not appear to show great need for bioaugmentation.

The field of bioaugmentation is rapidly expanding to include more wastewater, ex situ soil, and in situ groundwater applications, and a review of all these applications is beyond the scope of this white paper.
A large number of laboratory bioaugmentation studies have been conducted, and recent successes have been achieved in the field using bioaugmentation for in situ treatment of contaminated groundwater. As such, this paper focuses on bioaugmentation applications for in situ treatment of contaminated aquifers.

The remainder of this document presents a review of recently published laboratory and field studies investigating the performance of bioaugmentation for remediating contaminated groundwater in situ. The document focuses primarily on research and in situ applications for chlorinated solvents and MTBE. Section 2.1 of this report reviews pertinent laboratory investigations regarding the transport, biotreatability performance, survival, and monitoring of exogenous microbial cultures in saturated porous media. Section 2.2 summarizes findings from a variety of field applications, including demonstrations conducted at Dover Air Force Base, Kelly Air Force Base, the Bachman Road Residential Wells Site, the Naval Environmental Test Site at Port Hueneme, and the Schoolcraft Test Site. Section 3 summarizes the state-of-the-science regarding in situ bioaugmentation, and identifies principal research needs for further development of the technology.

2.0 CURRENT STATUS OF BIOAUGMENTATION

Bioaugmentation for aquifer restoration can be categorized as an innovative technology because while laboratory experiments and field applications at pilot scale have been successful, the numbers of full-scale applications are few. In fact, there have been no reported cases in the open literature where the technology has been used successfully to achieve site closure. As many aspects of the technology are still in the developmental stage, there is an abundance of on-going research studies investigating factors affecting the transport, survival, and performance of exogenous cultures in contaminated soil and groundwater. The number and diversity of field investigations continues to grow, involving a range of specialized cultures, reagent delivery systems, and experimental designs. In addition to new field investigations, continued data collection at older test sites (e.g., Dover AFB and the Schoolcraft Test Site) has provided an improved understanding of the parameters that control the success of in situ bioaugmentation applications.

2.1 Laboratory Research

The following sections summarize recent research regarding some of the key factors affecting performance of the technology, including microbial transport, substrate concentration and toxicity, species competition, substrate interactions. The development and application of new molecular tools for monitoring bioaugmentation performance is also described.

2.1.1 Microbial Transport

One commonly perceived limitation to bioaugmentation is the challenge of effectively distributing inoculated cultures throughout a contaminated plume. Depending on site-specific aquifer chemistry, many bacterial strains often sorb strongly to solids, and this can sometimes lead to clogging of injection wells and/or poor distribution of injected cultures. Major parameters that affect the distance across which bacteria cells are transported include bacterial cell surface properties (surface charge, hydrophobicity, physical structure), cell size, soil characteristics (macropore structure, grain size, organic content, clay type, etc.), groundwater chemistry (primarily ionic strength), flow rates, and additives. Proper engineering design can often minimize and/or avoid distribution and clogging problems by controlling injection rates and the concentration and type of bioremediation additives. A variety of techniques have been applied to improve microbial transport and distribution in aquifers, including use of surfactants, starvation of microorganisms, and development of bacterial strains that resist adhesion.

Subsurface microbial transport, as it pertains to bioaugmentation applications and microbial pathogen transport continues to be the focus of a variety of research efforts (Jennings et al., 1995; Burlage et al., 1995; Petrich et al., 1995; Camesano and Logan, 1998; Li and Logan, 1999; Major et al. 2002; Dybas et al. 2002; Steger et al. 2002; Schellenberg and Logan 2002). This section summarizes research regarding a variety of factors that affect microbial transport, including cell filtration and attachment, inocolum cell density and injection rates, cell surface characteristics, ionic strength, surfactants, filter bed surface roughness, and cell encapsulation. Although not

reviewed here, it is noted that cell starvation and aquifer matrix chemistry can also play an important role in affecting transport.

Filtration and Attachment of Injected Cultures

Understanding of microbial transport processes in the subsurface stems, in large part, from experience with sand and carbon filters used in the water and waste water treatment industry. Clean-bed filtration theory has been used for many years to describe colloid removal in packed-bed sand filters used for water treatment (Yao et al. 1971), and the theory has been extended to describe bacterial migration in sandy aquifers (Harvey and Garabedian 1991; Martin et al. 1992). A wide variety of research efforts have since demonstrated that bacterial migration and growth in groundwater is strongly impacted by filtration (including both attachment and detachment) (Ryan and Elimelech, 1996; Pang et al. 1998; Rogers and Logan, 2000).

Inocolum Cell Density and Injection Rates

Jennings et al. (1995) provided data that suggest that microbial transport through sandy aquifers is related to (1) injection pressure of the culture, (2) the influent substrate loading rates (e.g., the lower the substrate-loading rate the lower the transport of the culture), and nutrient injection. It appeared from these studies that continuous injection of nutrients causes biomass to accumulate or develop near the nutrient injection points.

Bourquin et al. (1997) studied the use of *Burkholderia cepacia* (previously *Pseudomonas cepacia*) $PR1_{301}$ to degrade TCE and DCE in a contaminated aquifer in Wichita, KS. The aquifer at the site is composed primarily of sands with hydraulic conductivities ranging from 115 to 225 m/day. The culture was grown to high concentrations (10^{12} cells/mL) and injected into the aquifer to achieve an in situ concentration of 10^9 cells/mL. PR1 was detected approximately 30 cm downgradient eight days after the culture was injected at 0.005% of the injected population density. Also, data indicated that the injection well became plugged during the bioaugmentation process. The culture was again added to the aquifer, but efforts were made to augment to a population density that would not cause biofouling of the injection well. Therefore, the culture concentration was started low and increased over time. Significant degradation of the contaminants (to non-detect levels) in the injection well was not achieved until the cell concentration became approximately 10^8 cells/mL. Again, plugging of the injection well was reported, and only minimal transport was achieved.

The effect of fluid velocity on the transport of motile and non-motile bacteria was studied by Camesano and Logan (1998). Radiolabeled cells were studied in saturated soil columns to determine the effect of fluid velocity on the transport of *Pseudomonas fluorescens* P17 (motile), P17 rendered non-motile, and *Burkholderia cepacia* G4 (non-motile) bacteria. Along with changing pore velocities, the ionic strength and cell concentration (both known to affect cell transport) were varied to determine the effects of these factors. As the pore velocity was decreased from 120 to 0.56 m/day, the fractional retention of motile cells decreased by 65%, while the predicted fractional retention for passive colloids increased by more than 800% over this same velocity range. Swimming cells were presumably able to avoid sticking to soil grains at low velocities, but at high velocities, cell motility did not reduce attachment. Increasing the cell concentrations of motile cells increased the overall retention of cells, which suggests that previously deposited cells provided a more favorable surface for bacterial adhesion than did the native soil. Increasing the concentration of non-motile cells (G4) resulted in lower retention. The authors believed that the deposited cells provide a less favorable surface for collection. The decrease in ionic strength decreased the retention of motile bacteria, but not to the degree that was observed for non-motile strains. The authors concluded that wider dispersal of cells during bioaugmentation might be achieved through the use of motile cells, low pumping rates, and low ionic strength.

Research has found that nonaqueous phase liquids (NAPL) in saturated porous media can affect microbial transport of certain bacterial species by increasing groundwater flow rates. Using column experiments, Rogers and Logan (2000) showed that the presence of tetrachloroethene (PCE) improved the mobility of *Pseudomonas fluorescens P17* in soil by a factor of 1.6. In contrast, NAPL had little measurable effect on migration of *P. putida KT2442* in soil. The researchers concluded that NAPL has the potential to increase cell transport by reducing effective porosity and increasing linear flow velocity.

Cell Surface Characteristics: Hydrophobicity, Electrophoretic Mobility, and Motility

Duston et al. (1997) studied the effects of bacterial cell characteristics on bacterial attachment and transport through porous media in a laboratory setting. Bacterial surface characteristics that appeared to affect attachment were electrophoretic mobility, hydrophobicity, and biopolymer production. Hydrophilic cells were observed to attach to hydrophilic surfaces according the DLVO theory. Size was found to influence retention of the cells in porous media with cells of diameters less than 0.1 μm and greater than 3.0 μm retained to greater levels than cells with diameters within this size range. Attachment of cells increased with increasing ionic strength, and attachment of cells to sand grains was greater than to glass beads. This difference was attributed possibly to the greater roughness of the sand grains. Also, lower hydraulic conductivities resulted in greater cell retention.

Hydrogenophaga flava ENV 735 offers promise as pure culture that can be used in bioaugmentation applications to enhance in situ aerobic biodegradation of MTBE and tert-butyl alcohol (TBA) (Hatzinger et al. 2001). However, initial laboratory studies with this species indicated that it did not move well through aquifer sediments. Streger et al. (2002) evaluated the transport characteristics of ENV735 in aquifer columns, and identified two strains of ENV735 with varying transport characteristics. One of the strains tended to sorb to aquifer solids and had poor mobility through the aquifer column, while the other strain was found to be adhesion-deficient and moved through the aquifer material much more readily. Consistent with the results of Dunston et al. (1997), varying cell surface characteristics were found to be responsible for the differing performance in migration for the two strains. Specifically, hydrophobicity and electrophoretic mobility were identified as primary factors affecting migration through the sand. Analysis by hydrophobic interaction chromatography determined that the sorbing strain was hydrophobic, while the adhesion-deficient strain was hydrophilic. This observation corroborates findings by DeFlaun et al. (1999), who also reported a positive correlation between enhanced cell transport and reduced hydrophobicity. Another difference between the two ENV735 strains was that the adhesion-deficient strain had no flagella, while a subpopulation of the sorbing strain was flagellated. Both ENV735 strains exhibited a strong affinity for anion exchange resin, which suggested that the mobility of these strains might be limited in aquifers where the aquifer particles have a negative surface charge. The isolation of the adhesion-deficient strain of ENV735 improves the potential for successful application of this culture for MTBE bioaugmentation applications. In addition, this work indicates that pure and mixed cultures used for bioaugmentation may include subpopulations with varying sticking efficiencies (adhesion).

While motile capability was not observed to enhance microbial transport in the work of Streger et al. (2002), the presence of a flagellum appears to improve microbial transport for other species. *Pseudomonas stutzeri* strain KC is a highly motile, flagellated rod-shaped bacterial strain that is able to degrade carbon tetrachloride (CT) without producing chloroform (Criddle et al. 1990). Strain KC grows well under both aerobic and denitrifying conditions, and degrades CT cometabolically in the presence of acetate (electron donor) and nitrate (electron acceptor). Under iron-limiting conditions, strain KC produces a small cofactor that can degrade CT in the absence of oxygen. Witt et al. (1999) used column experiments to show that strain KC is chemotatic toward nitrate and acetate (chemotaxis is the ability of motile microorganisms to bias their movement toward higher concentrations of chemoattractants). These experiments showed that certain exogenous cultures (e.g., strain KC) will migrate via chemotaxis, and this property can result in enhanced microbial transport during bioaugmentation.

Effect of Ionic Strength and Surfactants

Taylor and Hanna (1995) also observed a strong correlation between ionic strength and microbial attachment in saturated porous media. The attachment of *Methylosinus trichosporium* OB3b (a methanotroph) onto sand and sediments was markedly dependent on the ionic strength of the aqueous media. They found that an ionic strength of approximately 0.01 molal is needed to promote maximal attachment to the sand and sediments. In addition, the attachment process appeared not to be electrolyte-specific.

Li and Logan (1999) studied the effects of low ionic strength solutions and surfactants on the transport of bacteria in aquifers. The studies used radiolabeled microbes in microcolumns to study the effects of low ionic strength solutions (~0.01 mM), non-ionic surfactants (Tween 80, Triton 100 and 705, POE-10, and Brij + 35) and an anionic surfactant. The solutions reduced the sticking coefficients by an order of magnitude for natural soils. However, even with the improved sticking coefficients, the bioactive zones in aquifers were calculated to be approximately 1

meter. It was concluded that changes other than simple solution chemistry manipulations would be required to improve bacterial transport distances.

Streger et al. (2002) examined the effectiveness of surfactants for enhancing transport of the hydrophobic strain of ENV735. With the exception of the nonionic surfactant Tween 20, the surfactants tested were toxic to ENV735 at 0.01% (vol/vol) concentration, including Tween 80, Brij 35, JBR-425, sodium dodecyl sulfate, Igepal CO-720, Steol CS-330, and Tergitol 15-S-12. Tween 20 was observed to both enhance cell transport and the extent of MTBE biodegradation. The cause of the enhanced biodegradation was not determined.

Effects of Filter Bed Surface Roughness

Recently published work by Shellenberger and Logan (2002) provided empirical evidence that hydrophobicity and electrophoretic mobility alone is insufficient to predict bacterial adhesion. Using a series of filtration experiments with glass beads, this work found that for certain bacterial species, surface roughness can be an important factor in determining the extent of bacterial attachment to porous media. In this work, the perchlorate degrading strain KJ was filtered more efficiently on rough surfaces, while filtration of *E. coli* was not significantly different between smooth and rough surfaces. The authors concluded that the accuracy of filtration models might be improved by including a description of surface roughness and particle surface geometric effects. This work is consistent with the results of Dunston et al. (1997), who also reported greater cell attachment to sand grains relative to smooth glass bead surfaces.

Encapsulation of Injected Cells

Researchers have studied the effects of encapsulation on the transport rates of microbial cells (Petrich et al. 1995). The study involved an intermediate-scale (1- to 5-m length) tracer test in a field setting (a confined aquifer of silts, sands and gravels), where the transport rates of encapsulated-cell microbeads and other particles were tested. *Flavobacterium* ATCC 39723, a gram-negative aerobe, was encapsulated in agarose and the encapsulated bacteria were injected into the aquifer. The transport rate of the bacteria was then compared to those of aqueous and particulate tracers (1) bromide and (2) 2-, 5- and 15-μm-diameter Fluoresbrite plain polystyrene latex microspheres. The transport of the encapsulated cells was retarded relative to both the bromide and the polystyrene microspheres (only a few encapsulated cells were detected in the monitoring wells). The microscopy and gene probe screening were determined to be inadequate for the study, but the minimal detection of the encapsulated cells in the monitoring wells was attributed to a higher level of filtration and/or retardation.

Summary

Principal findings of the research reviewed above can be summarized as follows:

- Cell size can affect subsurface distribution. Bacteria typically have diameters of ~ 1 μm. Cells with diameters > 0.1 um but < 3.0 μm have been shown to migrate farther in porous media than cells with diameters outside this range.
- Wider dispersal of cells during bioaugmentation may be achieved through the use of motile cells, low pumping rates, and low ionic strength (e.g., < 0.01 molal).
- The hydrophobicity of specific microbial strains can affect their transport in the subsurface, but some pure cultures with desirable degradative capability possess adhesion-deficient subpopulations that are less hydrophobic than the parent culture. Isolation of adhesion-deficient strains offers the potential to improve distribution of injected cultures.
- For certain microbial species, cell motility and chemotaxic migration can improve cell transport through contaminated porous media.
- Certain surfactants can be used to reduce hydrophobicity and improve cell migration, but the effect is limited due to small radius of influence, and a variety of surfactants may be toxic to exogenous cultures. In addition, addition of surfactants may be problematic because it typically requires high doses to exert an effect on cell transport, can change soil properties, and may mobilize contaminants. As such, the benefit of surfactants for bioaugmentation has not been shown to have broad value. Specific surfactants may be beneficial for specific microbial strains.
- Cell attachment and retention is increased as the roughness the surfaces on aquifer material increases.

- Cell encapsulation with agarose has not been shown to improve cell transport, and may actually retard cell transport.
- Microbial transport in sand and gravel aquifers can be simulated as a function of clean-bed particle filtration, ionic strength, charge interactions and DLVO theory, and microbial growth and decay.

2.1.2 Substrate Concentration, Toxicity, and Source Area Applications

Pure phase, immiscible contaminant liquids and wastes (e.g., chlorinated solvents, and coal tar) pose one of the most vexing challenges to site remediation. Contaminant source areas at thousands of sites are characterized by the presence of a nonaqueous phase residual, commonly referred to as a dense, nonaqueous phase liquid (DNAPL). Difficult to remove because of their low aqueous solubility, high density, and high viscosity, DNAPLs persist at many RCRA, CERCLA, and DoD sites despite application of a variety of remediation technologies. The question of whether bioaugmentation offers potential to effectively remediate DNAPL source areas has only begun to be investigated at the field scale, but bench-scale evidence indicates that bioaugmentation with specialized dechlorinating cultures can significantly accelerate the rate of chlorinated solvent DNAPL dissolution.

Numerical fate and transport models developed in the early 1990's predicted that biodegradation could increase the rate DNAPL dissolution significantly above the rate that could be achieved by flushing alone (Seagren et al. 1994). Recent research provides empirical data that generally validates the underlying hypotheses of Seagren et al. and others. Biodegradation at the DNAPL/water interface can lower aqueous phase contaminant concentrations, thereby increasing the driving force for DNAPL dissolution and mass transfer. In addition, in the case of PCE, reductive biotransformation can facilitate dissolution because the daughter products of biotransformation (TCE, c-DCE, and vinyl chloride) all have much higher solubilities than PCE. Carr et al. (2000) demonstrated that tetrachloroethene (PCE) could be reductively dechlorinated to c-DCE in the presence of DNAPL (12% PCE in tridecane) in formate-fed reactors, resulting in a 14-fold increase in PCE dissolution from the DNAPL phase. Subsequent experiments in the same laboratory found that biodegradation by a dechlorinating culture enhanced chloroethene DNAPL dissolution by a factor of 5 to 6.5 times (Cope and Hughes 2001). Yang and McCarty (2000) observed that PCE DNAPL dissolution in a continuous-flow column increased about 5-fold when the column was bioaugmented with a dechlorinating culture. These studies indicate that biodegradation and bioaugmentation offer the potential to significantly reduce the longevity of chlorinated solvent DNAPLs in the subsurface.

Until recently, it was widely perceived that high substrate concentrations, particularly in the case of chlorinated solvents, would exert a toxic effect on exogenous and indigenous bacterial cultures and thereby inhibit microbial proliferation proximal to chlorinated solvent source areas (e.g., Huhling and Weaver, 1991). Laboratory research at Rice University (Hughes et al.) and Stanford University (Yang and McCarty) has now proven that high substrate concentrations are not inhibitory to certain types of bacteria that biodegrade chlorinated solvents. In fact, research suggests that certain dechlorinating microorganisms may thrive proximal to chlorinated solvent source areas because these organisms have a competitive advantage over other microorganisms in the presence of high chloroethene concentrations (Yang and McCarty 2000; Cope and Hughes 2001). Yang and McCarty (2000) found that high concentrations of PCE and c-DCE were inhibitory to methanogens and homoacetogens, and concluded that such inhibition is highly beneficial because it effectively reduces competition for electron donors, thereby making dechlorination at the DNAPL/water interface a more efficient process. Subsequent research by Yang and McCarty (2002) indicates that the efficiency of biologically enhanced PCE DNAPL dissolution is strongly affected by the type of electron donor used to provide reducing equivalents. In general, the efficiency of dechlorination correlates negatively with methane production.

At the field-scale, indigenous microorganisms have been observed to dechlorinate chloroethenes at concentrations well above 100 mg/L. Major et al. (1995) observed significant dechlorination activity in a shallow bedrock aquifer plume that contained TCE and c-DCE concentrations at 860 mg/L, and 430 mg/L, respectively. At the St. Joseph's Michigan site, natural reductive dechlorination activity was high despite TCE concentrations of 133 mg/L and c-DCE concentrations of 128 mg/L (Semprini et al. 1995). Exogenous cultures have also been applied successfully in situ to achieve effective dechlorination of chlorinated solvents at high dissolved phase concentrations. For example, bioaugmentation with the dechlorinating culture KB-1 achieved dechlorination of TCE to ethene in bedrock plume initially containing TCE > 100 mg/L (Chang et al. 2002).

In summary, the research described above has provided the following key findings:

- Certain dechlorinating microbial cultures (e.g., *Dehalococcoides*) may thrive and proliferate proximal to chlorinated solvent DNAPL source areas by biodegrading DNAPL constituents and outcompeting indigenous strains (e.g., methanogens).
- Chlorinated solvent DNAPL has been observed to be toxic to methanogens; a phenomena that is beneficial for bioaugmentation applications with *Dehalococcoides*.
- Provided sufficient electron donor, bioaugmentation with certain dechlorinating cultures can increase the rate of chlorinated solvent DNAPL dissolution by as much as 5 to 14 times. These laboratory studies suggest that bioaugmentation offers the potential to significantly reduce the longevity of chlorinated solvent DNAPL in the subsurface.
- Chlorinated solvent DNAPLs are not as toxic to bacteria as previously thought. However, it should be recognized that many chlorinated solvent plumes are comprised of complex mixtures of contaminants, and that specialized cultures may be inhibited by co-occurring contaminants. Substrate interactions can inhibit desired biodegradation via toxic inhibit or by enhancing growth of competing bacteria. These types of interactions are described further in the following sections.

2.1.3 Species Competition and Electron Donor Effects

It is reasonably well known that specialized cultures delivered to the subsurface for bioaugmentation applications may be subject to interference and competition by indigenous microflora. Recent research has focusing on the anaerobic bioremediation of chlorinated solvents has found survival of augmented cultures can be significantly affected by the type of electron donor that is applied. In a column experiment comparing the performance of pentanol, oleate, and olive as electron donors to support dechlorination of PCE DNAPL by a laboratory culture, Yang and McCarty (2002) observed extensive methanogenesis in the oleate- and pentanol-fed columns. In both cases, the increased methane production reduced PCE transformation. The authors noted that methane results from competitive substrate utilization by methanogens, and represents wastage in electron donor use (i.e., reducing equivalents are used for methane production instead of dechlorination). The data from this work suggested that, for certain types of electron donors, growth of methanogens can out compete the preferred strains (e.g., *Dehalococcoides*) if chlorinated solvent DNAPL concentrations are sufficiently low. These authors noted that methane production also creates the potential for aquifer clogging (via gas occupation of pore space). In addition, Yang and McCarty (2002) that use of insoluble electron donors for bioremediation and bioaugmentation applications poses several potential deleterious side-effects, including creation of excessively high dissolved organic carbon concentrations, elevated soluble iron and manganese, and sulfide production.

The addition of high electron donor concentrations for bioremediation and bioaugmentation applications creates elevated H_2 concentrations. Research has shown that dehalorespiring bacteria can use H_2 at lower concentrations than methanogens. Based on this observation, Fennell et al. (1997) and Yang and McCarty (2002) recommended bioremediation/bioaugmentation system designs that select for dechlorinating cultures, and minimize growth of methanogens.

Fennell et al. (1997) and Fennell and Gossett (1998) used a combination of laboratory experiments and modeling simulations to elucidate the relationship between hydrogenotrophic dechlorinating bacteria (e.g., *Dehalococcoides*) and methanogens. Laboratory experiments with a dechlorinating culture compared PCE dechlorination with varying electron donors (butyric acid, ethanol, lactic acid, and propionic acid). These experiments indicated that amendment with butyric and propionic acides resulted in less methanogenesis than did amendment with ethanol or lactic acid. Fennell and Gossett (1998) used a Michaelis-Menten type biokinetic model describe electron donor fermentation and competition for consequent hydrogen by methanogens and hydrogenotrophic dechlorinators. Calibrated to the laboratory data, the modeling results indicated that dechlorinators have a competitive advantage over methanogens at low H_2 levels. The model also predicted that adding excessive level of rapidly fermented, high H_2-level-generating donors will result in a dominant methanogenic population and eventual failure of dechlorination. In light of the empirical and modeling results, the authors concluded that differences in observed dechlorination for various electron donors was due to H_2-access thresholds and biokinetics that favored dechlorinators over competing methanogens at low H_2 concentrations (i.e., the dechlorinators examined were found to have a lower half-saturation constant than the methanogens).

2.1.4 Substrate Interactions and Effectiveness in Complex Mixtures

A variety of pure and mixed cultures have been developed that have been demonstrated to successfully destroy target pollutants (e.g., Harkness et al. 1999; Ellis et al. 2002; Major et al. 2002; Hristova et al. 2001; Hatzinger et. al 2001). While these cultures are often successful at treating target pollutants, the rate of biodegradation can sometimes be inhibited by the presence of inhibitory, co-occurring contaminants. Hughes and Parkin (1996a, 1996b) and Kaseros et al. (2000) documented the inhibitory effects present in mixtures of chlorinated ethanes, ethenes, and methanes. In the study of Kaseros et al. (2000), CT and CF inhibited PCE biodegradation, but the degree of inhibition was transient as a result of acclimation of the PCE-degrading community to CT and CF.

At many sites, MTBE occurs as a co-contaminant with other fuel hydrocarbons – principally benzene, toluene, ethylbenzene and xylenes (BTEX). Hanson et al. (1999) showed that the pure culture PM-1 could aerobically biodegrade MTBE rapidly, absent other competing substrates. Deeb et al. (2001) examined the ability of PM-1 to biodegrade PM-1 in the presence of BTEX in groundwater. Aqueous bioreactors without aquifer sediment were used for the experiment. The results indicated that 20 mg/L ethylbenzene or xylenes completely inhibited MTBE transformation by PM-1. Benzene and toluene were observed partially inhibit MTBE transformation by PM-1. The authors concluded that biodegradation of MTBE in many fuel hydrocarbon plumes might be delayed until MTBE has migrated beyond the BTEX plume. This work implied that the performance of bioaugmentation applications with specialized MTBE degrading strains might be inhibited by the presence of BTEX. However, the authors acknowledged the natural aquifer settings might behave substantially different than the laboratory experimental set-up. Specifically, the diversity of microflora in aquifers might have allowed for concurrent biodegradation of MTBE and BTEX, even under bioaugmented conditions.

2.1.5 Effect of pH on Culture Survival

Survival studies have indicated that factors such as pH affect the survivability of cultures (Dybas et al., 1995). Dybas et al. 1995 suggested that pH modification of the aquifer could produce a niche for the introduced culture. For the *Pseudomonas stutzeri* KC that they were investigating, the production of moderately alkaline conditions (7.9 to 8.2) was effective at creating such a niche and increasing the competitiveness of strain KC. The change in pH likely affected concentrations or speciation of metals (that inhibit growth of the cultured microorganisms) in the aquifer.

2.1.6 Molecular Monitoring Techniques and Culture Activity

In recent years, researchers have begun to apply sophisticated genetic analysis tools toward the problem of characterizing subsurface microbial communities and monitoring the bioaugmentation performance in the field. The development of molecular tools for mapping DNA sequences have allowed microbiologists to map the unique DNA signature of a variety of bacterial species, including strains that offer promise for bioaugmentation. Once the genetic signature, or fingerprint, of a specific strain has been characterized, environmental samples can be screened for the presence of that strain. The bacterial DNA in environmental samples can be harvested, amplified with polymerase chain reaction (PCR) analysis, and compared against known 16S rDNA sequences. Molecular analysis by PCR methods and 16S rDNA fingerprinting were often applied to detect the presence of pathogens in environmental samples. Now these molecular techniques are emerging as powerful tools for assessing bioremediation performance, the need for bioaugmentation, and bioaugmentation performance (Dybas et al. 1998; Loffler et al. 2000; Fennell et al. 2001; Hristova et al. 2001; Major et al. 2001; 2002; Lendvay et al. 2001; Hendrickson et al. 2002; Tani et al. 2002).

Chlorinated Ethenes

A variety of anaerobic bacteria are known to be capable of dechlorinating PCE and TCE to c-DCE, including *Desulfitobacterium*, *Dehalobacter restrictus*, *Desulfuromonas*, *Dehalospirillum multivorans,* and *Dehalococcoides ethenogenes* (Sholz-Muramatsu et al. 1995; Gerritse et al. 1996; Krumholz 1997; Maymo-Gatell et al. 1997; Holliger et al. 1998). However, members of the *Dehalococcoides* group are the only bacteria known to mediate complete dechlorination of PCE and TCE to ethene (Maymo-Gatell et al. 1997; Fennell et al. 2001). This finding has important implications for intrinsic and engineered bioremediation at sites contaminated with chloroethenes. Hendrickson et al. (2000; 2001; 2002) developed a PCR assay that uses species-specific primers to detect

Dehalococcoides in environmental samples. The species-specific primers were designed using the variable region sequences in 16S rRNA gene sequences (rDNA) from known dechlorinating bacteria found in Genbank. Hendrickson et al. used this PCR assay as a tool for characterizing the microbiology of bioaugmentation pilot test plots at Dover Air Force Base and Kelly Air Force Base. As further described in Section 2.2, the pilot tests at both these sites involved injection of dechlorinating cultures containing *Dehalococcoides* to achieve complete dechlorination of PCE and TCE to ethene. The application of the PCR assay by Hendrickson et al. (2000; 2001) confirmed that *Dehalococcoides* associated with the added culture had colonized the test plots at both sites, the effective dechlorination observed in both cases was, in all likelihood, attributable to the presence of *Dehalococcoides*. It is notable that at the Dover test site, *Dehalococcoides* was detected in the test plot three years after the pilot test terminated, indicating that the injected cultures can survive for long periods after injection into the subsurface.

Hendrickson et al. (2002) used PCR analysis and genetic sequence analysis to screen for the presence of *Dehalococcoides* 16S rDNA sequences in environmental samples from 24 sites contaminated with chlorinated ethenes. These researchers found that *Dehalococcoides* sequences were not detected at sites where dechlorination stalled at c-DCE. In contrast, *Dehalococcoides* was detected at sites where complete dechlorination to ethene was observed. This work established a strong correlation between the presence of *Dehalococcoides* and the extent of chloroethene biotransformation in groundwater plumes.

Loffler et al. (2000) described a two-step nested PCR method for detecting dechlorinating bacteria (*Desulfuromonas* and *Dehalococcoides*) in environmental samples. Specific primers directed against variable regions of the 16S rRNA genes of *Desulfuromonas* sp. strain BB1 and *Dehalococcoides* sp. strain FL2 were designed using Primer Selector (DNASTAR, Inc.) based on the nearly complete 16S rDNA sequences of strains BB1 and FL2. The nested PCR assay was performed using a universal bacterial primer set in the first step, followed by a second PCR that used specific primers for *Desulfuromonas* and *Dehalococcoides*. The method was tested on sediment samples from three rivers and six different chloroethene contaminated sites. *Dehalococcoides* was detected in samples from one of the chloroethene-contaminated aquifers and all three of the river sites. Microcosm studies were used to confirm the presence of PCE-dechlorination to vinyl chloride and ethene in samples where *Dehalococcoides* was detected by PCR assay. The work of Loffler et al. (2000) provided further evidence that the extent of chloroethene dechlorination in the subsurface environment is controlled, in part, by the presence of *Dehalococcoides*.

Additional evidence of the relationship between observed dechlorination in the field and the presence of *Dehalococcoides* was provided Fennell et al. (2001). Using various samples collected from a TCE-contaminated aquifer at Cape Canaveral Air Station (CCAS) in Florida, Fennell et al. (2001) performed microcosm studies and PCR analysis with *Dehalococcoides*-specific primers to investigate trends between observed dechlorination and the presence of *Dehalococcoides*. The microcosm studies investigated dechlorination response for a variety of electron donors, including acetate, propionate, and lactate. The results were consistent with those of Hendrickson et al. (2002) and Major et al. (2002): *Dehalococcoides* was only detected in samples that exhibited dechlorination of TCE to VC and ethene. In the sediments where *Dehalococcoides* was not detected, dechlorination of TCE did not occur over the 200 day monitoring period, despite an abundance of fermentative activity. The authors reported that the method detection limit for *Dehalococcoides* was 10^3 cells/0.5 g soil for direct PCR, and 5-10 cells/0.5g cells using a nested PCR approach. The work of Fennell et al. (2001) confirmed that *Dehalococcoides* occurs naturally at some TCE-contaminated sites, but that the distribution of natural *Dehalococcoides* strains can be very heterogeneous within any given site. In this work, the application of 16S rDNA-based PCR methods was a more sensitive measure than microcosm studies for detecting dechlorinating potential (some microcosms that tested negative for dechlorination also tested positive for *Dehalococcoides*). The authors emphasized that more development of PCR primer, probe, and array-technologies is needed before a simple, accurate test is available for widespread use.

Methyl Tert-Butyl Ether (MTBE)

Molecular monitoring has also emerged as powerful tool for assessing the need for bioaugmentation and bioaugmentation performance at sites contaminated with MTBE. Hristova et al. (2001) developed a highly-sensitive 16S rDNA-based PCR technique to enumerate the cell density of PM-1, an aerobic MTBE-degrader classified as a member of the *Rubrivivax gelatinous* subgroup of □-*Proteobacteria*. Laboratory experiments and analysis of field samples were performed as part of a bioaugmentation field trial evaluating performance of PM-1 to achieve in situ remediation of MTBE. The technique calculates a precise quantitative measure of a specific 16S rDNA sequence

from the initial exponential phase of the PCR. Using TaqMan PCR, the authors reported that PM-1 had survived at least 7 months after injection into the test plots. In laboratory trials, PM-1 cell densities (as estimated by TaqMan PCR) increased as the culture degraded MTBE.

The sensitivity of in situ PCR analysis and DNA-fingerprinting was compared against fluorescent in situ hybridization (FISH) in a field bioaugmentation trial conducted by Tani et al. (2002). The cometabolic TCE-degrader *Ralstonia eutropha* KT1 was injected into a TCE-contaminated aquifer and toluene was provided as a primary substrate. Seven thousand (7000) liters of cell suspension was injected, and groundwater samples were collected within a short radius of the injection well over a period of 80 days. Samples were collected and analyzed for *R. eutropha* by both in situ PCR targeting the phenol hydroxylase gene and FISH by targeting 16S rRNA. In general, the results suggested that in situ PCR was a more sensitive method for detecting *R. eutropha*.

Chloroethanes

Sun et al. (2002) reported the isolation of strain TCA1, a halorespiring anaerobic bacterium that reductively dechlorinates 1,1,1-trichloroethane (TCA) to 1,1-dichloroethane (DCA) and chloroethane. Strain TCA1 can grow with hydrogen or formate as electron donors. Laboratory bioaugmentation experiments that used sediments from the Bachman Road and Schoolcraft sites confirmed that the culture could be delivered to treat TCA in groundwater. The study noted that Strain TCA1 did not dechlorinate 1,1,1,2-tetrachloroethane, 1,1,2-trichloroethane, 1,2-dichloroethane, 1,2-dichloropropane, PCE, or TCE when they were added as potential electron acceptors. Nearly the full length of the 16S rDNA sequence of strain TCA1 was mapped and compared with available 16S rDNA sequences to characterize the phylogeny of the microorganism. Strain TCA1 was found to be closely related to *Dehalobacter restrictus*. This work indicates that bioaugmentation offers promise for remediation of TCA and DCA contaminated sites, and that molecular monitoring tools are available for monitoring the performance of TCA1 in bioaugmentation applications. This work also illustrates the specificity of a culture developed for bioaugmentation (the culture was incapable of dechlorinating a variety of other chloroethanes and chloroethenes).

Summary

Key findings of research regarding molecular monitoring techniques for bioaugmentation can be summarized as follows:

- DNA-fingerprinting techniques that were once only used to characterize the phylogeny of pure cultures are now being applied at the field scale for bioaugmentation monitoring purposes.
- 16S rDNA sequences have been mapped for a limited number of strains known to achieve desired biodegradation reactions in the field. Use of this genetic information has made it possible to detect and monitor the survival and persistence of certain specialized cultures during bioaugmentation applications.
- To date, 16S rDNA methods have been developed to detect specialized strains that degrade certain chloroethenes, chloroethanes, and MTBE.
- Molecular monitoring has been used to evaluate the need for, as well as performance of, bioaugmentation.
- Through the development and application of 16S rDNA-based PCR methods, microbiology (presence/absence of requisite strains) has been shown to be a key design parameter that governs the performance of enhanced bioremediation approaches for treating chloroethenes in groundwater.
- In some cases, molecular monitoring may be a more sensitive measure of intrinsic biodegradation potential at a given site.
- There is a strong need for continued development of molecular monitoring technology and continued genetic mapping of sequences of cultures known to mediate desirable metabolic reactions.

2.2 Field Testing and Application

The application of bioaugmentation for aquifer restoration is an innovative technology that only recently has had successful field demonstration. These successes have stimulated interest in the potential of the technology and investments have been made to evaluate this potential. While information on successful demonstrations is limited, several case histories have been reported, and the table below contains a list of bioaugmentation projects that are currently being conducted or have been completed for site remediation. The list provides the location of the project, scale of the application (i.e., pilot-scale or bench-scale), the type of contamination, the culture being used, obstacles

encountered during the project, and the outcome of the project. This table does not provide an exhaustive list of bioaugmentation projects in part due to the business-sensitive or preliminary nature of many of the projects being performed. The list presented below focuses primarily on projects that have been described in peer-reviewed journals and proceedings of technical conferences.

Site Name, Location	Scale of Project	Type of Contamination	Culture being Augmented	Obstacles Encountered	Outcome of Project
Dover Air Force Base Dover, DE	Pilot-scale	Chlorinated Solvents	Pinellas culture	Culture may have been distributed into control section of test plot .	Success
Gilbert-Mosley Site Wichita, KN	Pilot-scale	Chlorinated Solvents	*Burkholderia cepacia*$_{301}$	None identified.	?
Chico Municipal Airport Chico, CA	Pilot-scale	Chlorinated Solvents	*Methylosinus trichosporium* OB3b	Short-term effectiveness, culture survivability.	?
Carbon Tetrachloride Site Schoolcraft, MI	Full-scale	Carbon Tetrachloride	*Pseudomonas stutzeri* KC	Required modification of groundwater pH; Chloroform and sulfide accumulated if electron donor dose was too high. Control plot not included – as such, relative contributions of exogenous and indigenous cultures cannot be known for sure.	Success
Industrial Site, Pennsauken, NJ	Pilot-sale	Chlorinated VOCs	*Burkholderia cepacia* ENV435	None identified.	?
Fallon Naval Air Station Fallon, NV	Bench-scale	Chlorinated Solvents	Pinellas culture	High groundwater SO_4, High pH, High TDS, and High metals concentrations, bioaugmentation did not stimulate complete dechlorination	Pilot test not initiated due to microcosm results
US Naval Hydrocarbon Naval Test Site, Port Hueneme, CA	Pilot-scale	MTBE	MC-100 culture	Confounding factors: native bacteria degraded MTBE, and oxygen delivery method prevented accurate determination of biodegradation rates.	Ambiguous result
Kelly AFB, San Antonio, TX	Pilot-scale	Chlorinated Solvents	KB-1 culture	None identified	Success
Bachman Road, Lake Huron, MI	Pilot-Scale	Chlorinated Solvents	Bachman Road Culture	Presence of indigenous dechlorinating culture contributed to a portion of the dechlorination – control plot did not receive electron donor	Qualified success: treatment very effective; but relative roles of exogenous and indigenous cultures not distinguishable
Aerojet Gen. Corp., Sacramento, CA	Large Pilot – Scale	Chlorinated Solvents	KB-1 culture	None	Success

2.2.1 Dover Air Force Base, Dover, Delaware

Bioaugmentation was successfully implemented on a TCE-contaminated aquifer at Dover AFB, Delaware, using a microbial enrichment culture from NAS Pinellas, Florida (Ellis et al. 2000). The Pinellas culture has the capability to completely transform TCE to ethene (Deweerd et al., 1999, Harkness et al. 1999), and is known to contain *Dehalococcoides ethenogenes* (Hendrickson et al. 2001). The pilot test employed a closed-loop, recirculatory flow system that consisted of three extraction wells, three injection wells, and a series of performance monitoring points in between. The surface area of the pilot system was 12 meters by 18 meters, and the bottom of the 15-foot-deep cell was placed into the aquitard at the site.

The need for bioaugmentation was established prior to the start of the pilot test. Prior to the pilot test, the average TCE and c-DCE concentrations were 4,800 and 1,200 µg/L, respectively. Data indicated that the aquifer was naturally aerobic, and not conducive to complete reductive transformation of TCE and c-DCE. Laboratory microcosm studies completed prior to the pilot test to assess the ability of the indigenous microorganisms to dechlorinate TCE. Static microcosm were incubated and sampled over the course of six months to evaluate the relationship between TCE biotransformation and type of organic electron donor (butyrate, benzoate, acetate, ethanol, and molasses were evaluated). A separate set of microcosms were fed lactate biweekly to evaluate TCE under semi-continuously amended conditions. None of the amendments tested were able to promote dechlorination past c-DCE (vinyl chloride and ethene were not observed) during six month trial. These results are consistent those of Harkness et al. (1999), who found that the microorganisms in Dover sediment were incapable of dechlorination past c-DCE over the course of 371 days. Ellis et al. (2000) concluded that ethene-forming microorganisms were absent or could not be stimulated under a wide range of conditions. In an additional set of experiments, Harkness et al. (1999) demonstrated that bioaugmentation with the Pinellas culture achieved reproducible and reliable dechlorination of TCE to ethene in Dover sediments.

The field pilot system operated by extracting groundwater from the three downgradient wells, blending the extracted water, adding bioremediation amendments, and reinjecting the water into three upgradient wells. The groundwater and pumping rate of 11.6 L/min created a groundwater residence time of 60 days within the test area.

Prior to bioaugmentation, the pilot test area was conditioned with electron donor and nutrients to achieve reducing conditions suitable for proliferation of the Pinellas culture. During this initial phase, electron donor (100 mg/L lactate) was delivered on a 7-day pulsed feeding schedule to minimize biofouling in the injection well. Lactate was fed into the injected water for 3.75 days, followed by a 0.25 flush of unamended groundwater. A nutrient mix consisting of 5 mg/L ammonium and 5.5 mg/L phosphate were then fed into the flow stream for 2.75 days. After 269 days of operation in the non-augmented configuration, dechlorination did not proceed past c-DCE. However, most of the TCE had been reduced to c-DCE, by 269 days.

After 269 days of substrate/nutrient injection, 180 L of the Pinellas culture was injected into the test system. On day 284, another 171 L of aqueous culture was injected into the system. The production of vinyl chloride was detected 91 days after the first episode of bioaugmentation. The appearance of ethene occurred shortly after the appearance of VC. The ratio of VC and ethene to c-DCE continued to increase through the remainder of the demonstration. By day 479, reductive dechlorination was stoichiometrically degrading TCE and c-DCE to levels below EPA maximum contaminant levels (MCLs) without the production of toxic by-products. By day 509, TCE and c-DCE were fully converted to ethene. Mass balance analysis indicated that 75 to 80% of the original concentrations of TCE and c-DCE were recovered as ethene.

After the completion of the pilot test, Hendrickson et al. (2001) used 16S rDNA-based PCR methods to screen for the presence of *Dehalococcoides* within the test plot. One year after the completion of the pilot, *Dehalococcoides* was detected throughout the test plot, but was not detected outside the pilot test area, which provided additional evidence that the attainment of complete dechlorination within the test plot was linked to the presence of *Dehalococcoides*. Additional sampling performed two and three years after the completion of the pilot detected the continued presence of *Dehalococcoides* within the pilot test area, but again not in the upgradient background wells. These data indicate that the *Dehalococcoides* strains within the Pinellas culture can survive for a long period after injection into the subsurface, and continue to dechlorinate as along as the required anaerobic environment is maintained.

2.2.2 Gilbert-Mosley Site, Wichita, Kansas

Camp Dresser and McKee (CDM) conducted a pilot-scale demonstration employing an aerobic bacterium to treat groundwater contaminated with chlorinated aliphatic hydrocarbons including TCE, dichloroethene (DCE), vinyl chloride (VC), and perchloroethylene (PCE) at a site in Wichita, Kansas (Bourquin et al.1997; CDM, 1998). The site was contaminated by 50 years of industrial activity and the contaminant plume had grown to over 2,000 acres. The city of Wichita investigated the potential for bioaugmentation to remediate the plume at reduced costs over conventional pump-and-treat methodologies.

Burkholderia cepacia $PR1_{301}$ is a bacterial strain that constitutively produces dioxygenase enzyme, even in the absence of an inducer such as toluene or phenol. This specific strain was isolated in the laboratory by EPA. The organism had been shown to cometabolically degrade CAHs in laboratory tests but was not previously tested in a field situation.

A pilot-scale biobarrier was designed and installed at the Gilbert-Mosley site in Wichita. Oxygen and the microbial culture were continuously injected into the aquifer to form a barrier to degrade contaminants as they passed through the active zone. The concentrations of CAHs were reduced from approximately 500 parts per billion (ppb) to below detection within 24 hours. CDM estimated an initial degradation rate of 94.5 μg/mL/hour.

The positive results from the pilot test led to the decision to proceed to full-scale application. CDM estimated that the cost savings using bioaugmentation in place of a pump-and-treat system at this site would save the city between $7 and $10 million. The full-scale system was never completed, since during the design process, the city determined that the plume was being contained by natural barriers and engineered treatment was not necessary.

While this was one of the first field demonstrations of a successful field application of bioaugmentation for aquifer restoration purposes, the amount of information available in the open literature leaves some questions as to the actual effectiveness of the application. Because the site appears to have been sparged and culture fluids were continuously injected, and because no control data were presented, it is not clear that the removal was due to the addition of the culture. In addition, the observation that PCE was degraded aerobically has not been demonstrated elsewhere. Still, the demonstration was declared a success, and in 1997 the American Academy of Environmental Engineers presented CDM an award of excellence for their effort.

2.2.3 Chico Municipal Airport, Chico, California

Researchers from Lawrence Livermore National Laboratory (LLNL) conducted a field test of an in situ biofilter employing a methane utilizing bacterium (methanotroph) in a TCE-contaminated aquifer at the Chico Municipal Airport in Chico, California (Duba et al. 1996). The plume at this site is approximately 500 meters wide by 2,000 meters long with a maximum TCE concentration between 1.0 and 1.5 ppm. The water table is at approximately 26 meters bgs. The plume was restricted to the Tuscan formation, which is characterized as a heterogeneous mix of cobbles and finer-grained materials. The porosity was estimated at 40% with a permeability of $3\mu m^2$ and a groundwater velocity of 30 cm/day. Aquifer testing and geochemical analyses showed that the site was suitable for application of the methanotrophic bacterium *Methylosinus trichosporium* OB3b.

Methylosinus trichosporium OB3b has been studied extensively in the laboratory and is known to cometabolically degrade TCE when supplied with methane as the primary substrate. Two 1000-L batches of culture were grown in the laboratory in a 1,500-L fermentor, then centrifuged to a paste, bottled, and shipped to the site on ice. The cells were suspended in TCE-free groundwater to a density of approximately 5.4×10^9 cells/mL, and buffer and tracer were added. Approximately 1,800 liters of the suspension were injected into a single well at approximately 3.8 L/min. No primary substrate was added with the injected culture. Immediately following injection of the culture, 400 liters of uncontaminated groundwater was injected into the well to move the culture and distribute it in the aquifer. Groundwater was then extracted from the well at 3.8 L/min for 30 hours, then at 2.0 L/min for the duration of the test.

Groundwater samples were collected from the extraction well and two monitoring wells located approximately 1 meter from the extraction well. The samples were analyzed for TCE concentrations, bacterial enumeration, and tracer concentrations. The results showed that approximately 50% of the injected bacteria were retained by the

aquifer and were presumed to have attached to the sediment. Over the first 50 hours, TCE concentrations were reduced by 98% from 425 ppb to less than 10 ppb. The performance gradually decreased with TCE concentrations in the extracted water increasing to background levels after 40 days.

This demonstration showed that the injected culture was able to degrade TCE for a limited period of time. The culture did not appear to survive but it was unclear if this was due to the lack of a primary substrate or inability of the bacterium to compete and persist in the formation. Predation was ruled out based on the relative numbers of protozoans and *M. trichosporium* cells that were recovered in groundwater that was extracted after 39 days. Regardless, the researchers recognized that the sustainability of the performance needed to be extended beyond the 2 days and that heterogeneity in the subsurface would have an impact on the ability to create an in situ biofilter.

2.2.4 Carbon Tetrachloride Site, Schoolcraft, Michigan

Researchers at Michigan State University conducted a full-scale field demonstration of bioaugmentation in an aquifer contaminated with carbon tetrachloride (CT) and nitrate (Hyndman et al. 2000; Dybas et al. 2002). The demonstration evaluated the performance of bioaugmentation in a biocurtain system designed to intercept and treat the downgradient edge of CT plume. *Pseudomonas stutzeri* KC was selected for the test because of its known ability to degrade CT without producing chloroform (CF). The requirements for CT transformation by strain KC are (1) adequate concentrations of nitrate and electron donor, (2) anoxic denitrifying conditions, (3) iron-limited conditions, and (4) trace levels of copper. In addition, CT transformation by strain KC is optimal at a pH ~8.

The aquifer at the Schoolcraft test site consists of 27 m of fine- to coarse-grained sands from glacial outwash, with a water table approximately 5 m bgs, and an average groundwater flow velocity of 15 cm/day. Hydraulic conductivity values range from 1.1×10^{-1} to 4.0×10^{-3} cm/s. The CT plume (Plume A) is approximately 1.6 km long and 160 m wide, with an average CT concentration of ~ 30 ppb. A pilot study performed at the site previously demonstrated that CT transformation (60 to 65% removal efficiency) could be achieved in situ through inoculation with strain KC, addition of acetate and phosphate, and pH adjustment (Dybas et al. 1998). The pilot study also found that CF generation occurred in regions where strain KC activity low, and uniform CT transformation was not achieved because of inadequate hydraulic control. The full-scale system was designed using data and design parameters collected from the pilot test, aquifer characterization, laboratory studies, and three-dimensional solute transport modeling.

The full-scale bioaugmentation/biocurtain system was installed at the leading edge of the plume, in a linear array of 15 adjacent injection/extraction wells aligned perpendicular to the natural groundwater flow gradient. Each well alternatively served as either an injection or extraction well during different operational phases. The full-scale biocurtain was approximately 15 m long. The primary bioremediation additives used were acetate (electron donor), sodium hydroxide (pH adjustment), phosphate (nutrient), and strain KC. An above ground chemical addition system was designed to add tracer, adjust pH, and provide weekly delivery of substrates and nutrients to the biocurtain. System performance was assessed in a series monitoring wells installed upgradient and downgradient of the biocurtain. PCR techniques were applied to track the extent of migration of strain KC downgradient of the biocurtain.

The demonstration was performed in seven primary phases: (1) aquifer characterization and system installation (days 1 - 52); (2) tracer testing with bromide and fluorescein to assess solute transport between delivery wells and downgradient monitoring points (days 53 – 72) ; (3) pre-inoculation mixing and adjustment to pH 8.2 (days 73-116); (4) inoculation and feeding (days 117 – 199); (5) re-inoculation and feeding (days 200-313); (6) feeding with reduced acetate concentrations (days 314 to present); and (7) solid-phase evaluation of contaminants and microbes (days 336-342 and 1006-1013).

During a typical delivery event, a combined flow rate of 150 L/min groundwater was extracted from alternating delivery wells, circulated through the chemical addition/mixing system, and then injected into adjacent delivery wells. On day 117, the biocurtain was inoculated with 18,900 L of strain KC culture (~10^7 cfu/mL) that was grown aerobically on site. Thereafter, the delivery system was operated weekly for a 5 hour period to deliver acetate (100 mg/L), phosphate (10 mg/L), and alkali (adjust pH to 8.2). Delivery of bioremediation additives was followed by a 1 hour reversed flow operation to reduce biofouling at the well screen. On days 200 and 201, half of the delivery well gallery was re-inoculated with 37,000 L of strain KC culture grown (~10^7 cfu/mL) with reduced acetate

concentration and added trace metals. The decision to reinoculate was based, in part, on the limited detection of strain KC downgradient of the monitoring wells. On day 314, acetate levels were reduced to 50 mg/L to prevent sulfate reduction.

Dybas et al. (2002) reported that sustained and efficient (98%) removal of CT has been observed in the biocurtain system for over 4 years. Transient levels of CF and H_2S were observed, but both disappeared when the concentration of acetate in the feed was reduced from 100 to 50 mg/L. Denitrification was stimulated by addition of acetate and strain KC, and nitrate levels were reduced to below drinking water standards at both acetate doses. Cell migration after the first inoculation appeared limited, and the investigators suggested that much of the strain KC culture might have been attached to sediments close to the point of injection. Nine days after inoculation, strain KC and tracer were detected 1 m downgradient of the delivery well gallery, indicating that some cells had travel at least as fast as the advective groundwater velocity. The culture was also detected at a few locations 2 m downgradient of biocurtain. Subsequent monitoring, however, indicated that the initial inoculation achieved adequate colonization of the biocurtain area. Fifty-three days after the northern half of the biocurtain was re-inoculated, strain KC was detected at all sampled locations, including locations downgradient of the southern half of the biocurtain.

The Schoolcraft project represents what may be the longest-sustained successful bioaugmentation application to date. Based on the absence CF over most the demonstration, and the apparent colonization and growth of strain KC, authors concluded that augmentation with strain KC was the principal mechanism for treating CT in the site groundwater. However, the authors also acknowledged that indigenous microorganisms may have also contributed significantly to the degradation of CT. Since no control plot was operated during the demonstration, the relative effects of strain KC and the indigenous microflora cannot be known for certain. In any case, the project demonstrated the feasibility of pulsed-pumping operation for achieving effective treatment with low-pumping volumes and short pumping durations. Except for the weekly 6 hour period of reagent delivery and groundwater recirculation, the system was turned off and the biocurtain operated passively to treat contaminated groundwater.

2.2.5 Industrial Site, Pennsauken, New Jersey

Envirogen, Inc., conducted a field evaluation of bioaugmentation for treating chlorinated solvent contamination using a strain of *Burkholderia cepacia* (Stephan et al., 1999). The test was conducted at an industrial facility where the groundwater was contaminated with 1,000 to 2,500 μg/L of chlorinated solvents, including TCE, DCE isomers, and vinyl chloride. The aquifer formation was described as heterogeneous, consisting of silty-fine to medium-grade sand interspersed with thin lenses of gray clay. A pilot-scale test system that included both control and test plots was installed in a higher permeability layer confined between two clay lenses. Each plot was approximately 12-m in length and contained a set of three nested injection wells, three rows of three nested monitoring well clusters, and a recovery well. Two single monitoring wells were installed at each end of the test plot and one additional nested monitoring well was placed between the two plots.

A variant of *Burkholderia cepacia* $PR1_{301}$ was isolated for its adhesion-deficient properties and identified as *B. cepacia* ENV435. The culture was grown in the laboratory in 550 liters of basal salts medium by feeding it alternating batches of sucrose and phenol. The culture was transferred into a 1,100-L plastic tank and shipped to the site, then transferred to holding tanks on site for injection.

Two modes of injection were used. For the first injection, the culture was added to groundwater extracted from the end of the test plot, then recirculated through the injection wells at the head of the test plot. The culture was added to achieve approximately 1×10^{11} cells/mL. During the second injection, the culture was injected directly into the monitoring wells under pressure, then the monitoring wells were cleared using pressurized oxygen. During the evaluation, microbial transport, oxygen distribution, and VOC reductions were monitored.

Microbial transport was evaluated during the first phase of injection based on the recovery of colony forming units (CFU's) on plates containing antibiotics against which the injected strain was resistant. The time required to reach the peak of the recovery curve was used to estimate a linear velocity for the cells, which was compared to a conservative bromide tracer. The resulting velocities were combined to calculate a ratio showing the relative movement of the cells to the movement of groundwater. The resulting Br:ENV435 ratios were between 1.26 and 1.43, suggesting that the microbes were easily transported. The recovery data was not as promising since the percentage of cells recovered was much less than expected based on the survivability observed during microcosm

testing. This suggested that a large number of cells were either being filtered out or were not surviving the in situ conditions. A half-life of 1 to 2 days was estimated using first order decay analysis. The unexpected loss of cells led to the second mode of injection, which was an attempt to distribute a sufficient population of ENV435 to degrade the VOCs. It was not possible to monitor the distribution of the cells over distance from the monitoring wells.

During the first injection, the oxygen was depleted rapidly within 2 meters from the injection point. During the second run, pure oxygen was injected into the monitoring points. The DO in the groundwater was raised to 20 mg/L and declined to greater than 2 mg/L over a 3- to 5-day period.

During the first phase of injection, VOC concentrations showed a marked decrease in the test plot compared to the control plot. While VOC concentrations in the injected water varied over time, the concentrations in the test plot were consistently lower than the injected concentrations. Total VOC concentrations dropped from approximately 2,200 μg/L to below 500 μg/L at most monitoring locations with most of the reaction occurring within two meters from the point of injection. The ratio of VOCs degradable by *Burkholderia cepacia* to compounds, which this organism cannot degrade (i.e., (TCE+DCE+VC)/(PCE+DCA+TCA)), decreased over time suggesting that biotransformation was primarily responsible for the observed reduction in concentration rather than abiotic mechanisms. The VOC concentration in the control plot remained relatively constant over the test period.

During the second phase of injection, the concentration of VOCs was reduced to as low as 50 μg/L suggesting that some benefit had resulted from the additional injections of the culture and the injection of oxygen across the treatment zone. As with Phase 1 injection, the ratio of degradable VOCs to nondegradable VOCs decreased over time, which suggests that biodegradation was the predominant removal mechanism.

The results from the above evaluation show the potential for bioaugmentation at this site after site conditions were altered slightly to favor survival of the added bacterium. They also point out some of the problems associated with the distribution of microorganisms in the subsurface. A variant of *B. cepacia* $PR1_{301}$ was selected based on its adhesion-deficient properties, but still it was effectively removed in the aquifer. The fact that most of the chlorinated solvent removal occurred within the first 2 meters could have been caused by insufficient oxygen, insufficient population of ENV435, lack of dioxygenase expression by the microbes, or any combination of the three. The second-phase injection, where culture and oxygen were added at discrete points, did not answer these questions. The results observed suggest that a biobarrier or in situ biofilter application may be more appropriate than large-scale distribution of this organism within the aquifer.

2.2.6 Naval Air Station, Fallon, Nevada

Naval Air Station Fallon was selected as a site to demonstrate the ability of the consortium (Pinellas culture) isolated from NAS Pinellas to degrade chlorinated ethenes. No published information regarding the bioaugmentation project at NAS Fallon is available. The test site is adjacent to a former unlined fire training area that was contaminated primarily with fuels, but also low levels of chlorinated ethenes (maximum PCE concentrations of 100 µg/L). The training area was in use from the 1950s to 1988. The site contains five separate test lanes that remain from another bioremediation project. These lanes are oriented parallel to the groundwater flow direction and are hydraulically isolated to allow for comparison of five different testing scenarios.

Although chlorinated ethene concentrations are present at sufficient levels, and the test lanes would need minimal modification to perform the tests, the site presents several obstacles for reductive dechlorination through bioaugmentation. The groundwater contains high total dissolved solids, very high sulfate concentrations (7,200 mg/L), and high levels of metals that may be toxic to microorganisms (e.g., arsenic, molybdate, borate).

Microcosm experiments were prepared in 100-mL bottles using soil and groundwater from the site that had been initially sparged with nitrogen to remove the chlorinated and maintain anaerobic conditions. The bottles were then amended with PCE to achieve a concentration of 40 µM. The microcosm experiments were designed so that two electron donors (lactate and methanol), supplemental nutrients, and the Pinellas culture were added to the microcosms alone and in combination. The experimental matrix included two sets of microcosms where electron donors alone were added, two sets of microcosms where electron donors and nutrients were added together, and two sets of microcosms where electron donors, nutrients, and an active Pinellas culture were added, along with

unamended, killed, and positive controls. Electron donors were added weekly at concentrations of 5.0 mM sodium lactate and 10.0 mM methanol. Nutrient-amended microcosms received a revised formula of anaerobic mineral medium consisting of potassium and ammonium salts, trace metals, and a vitamin mix. This medium was added only at the beginning of the test.

The methanol-fed microcosms (both the unaugmented and augmented microcosms) quickly demonstrated slow dechlorination of PCE to TCE without significant reduction of sulfate (methanol is not used extensively by sulfate-reducing bacteria). Throughout the 22-day experiment, dechlorination beyond TCE was insignificant.

PCE concentrations in the lactate-fed microcosms (unaugmented and augmented) remained near the starting concentrations for approximately 19 weeks. The majority of the lactate in both the bioaugmented and non-bioaugmented bottles was used to reduce sulfate. Sulfate was reduced at a relatively constant rate of approximately 450 mg/L/week for the first 13 weeks. At week 16, sulfate had been depleted in several of the lactate-fed bottles, yet no significant dechlorination was occurring. It was then determined that the high sulfide concentrations (1,000 mg/L) in the microcosm bottles may have been inhibiting the reductive dechlorinating microorganisms. During week 19, ferrous chloride was added to the lactate-fed microcosms to precipitate the sulfide, and the sulfide concentrations were reduced to <5 mg/L in the bottles. Immediately following the ferrous chloride addition, the PCE concentrations declined at significant rates and both acetylene and ethene were produced as end products. After 12 weeks of the ferrous chloride addition, nearly all of the PCE had been removed from the microcosms and acetylene/ethene concentrations increased to 20 to 30 μM in all of the augmented and non-augmented bottles. The production of acetylene was believed to be the product of a non-biological reaction catalyzed by the iron sulfide produced during the ferrous chloride addition. Additionally, insignificant difference in the PCE reduction rate between the bioaugmented and non-augmented bottles suggests that the Pinellas culture will not greatly affect the reductive dechlorination rate or extent at the NAS Fallon site. Therefore, field-scale bioaugmentation activities were not pursued at this site.

2.2.7 USN Hydrocarbon National Environmental Test Site, Port Hueneme, California

Researchers at Equilon Enterprises (Shell/Texaco) and Arizona State University conducted a pilot-scale field demonstration of bioaugmentation to treat an MTBE-contaminated aquifer at the U.S. Naval Hydrocarbon National Environmental Test Site at Port Hueneme, California (Salanitro et al. 2000). Contamination at the site is the result of leaking tanks and piping at the Navy Exchange (NEX) service station. The dissolved MTBE groundwater plume extends over 1,500 m from the source with concentrations of 2,000-9,000 μg/L in the test plots. The water table is approximately 3 m bgs and the thickness of the upper aquifer is 3 m. The groundwater velocity ranges between 0.03 and 0.15 m/day.
This demonstration used the microbial consortium MC-100, which is known to degrade MTBE to CO_2, to assess the performance of oxygenation and bioaugmentation in a barrier design

Laboratory microcosm studies were performed prior to the initiation of the field demonstration in order to assess the relative MTBE biodegradation performance of MC-100 and the indigenous aquifer bacteria. Following a 2 to 3 week lag period, MTBE was biodegraded at an apparently zero order rate (254 μg/L day^{-1}) to nondetectable levels in aerobic unamended, groundwater microcosms within 63 days. In groundwater microcosms augmented with 10 to 12 mg/L MC-100, MTBE was biodegraded to nondetectable levels within 2 to 3 weeks. In general, the rate of MTBE removal in the bioaugmented microcosms was 3 to 5 times faster relative to unamended microcosms. These studies provided evidence that the indigenous bacteria were capable of biodegrading MTBE. The authors hypothesized that the indigenous microorganisms were not able to biodegrade MTBE as quickly as the MC-100 culture. At the same time, however, they provided data that suggested that cell density was the primary cause for the difference in biodegradation rates between the augmented and unamended microcosms. In an additional set of microcosms that received 5 mg/L of MC-100, the lag period and rate of MTBE biodegradation was not significantly different than in unamended microcosms.

Three different plots were operated during the field demonstration: (1) a control plot (without treatment), (2) a biostimulation plot with oxygen injection only, and (3) a bioaugmentation plot with oxygen injection plus augmentation with MC-100. Each plot was approximately 6 m wide by 15 m long and aligned parallel the groundwater flow direction. An array of monitoring wells was placed both above and below each of the test plots.

Both test plots were oxygenated for 7 weeks prior to augmenting the bioaugmentation plot with MC-100. Oxygen gas delivery was intermittent, with a total of about 1700 L of O_2 delivered to each plot 4 to 8 times per day. Dissolved oxygen concentrations increased in the biostimulation and bioaugmentation plots by 5 to > 20 mg/L within a few weeks of oxygen gas injection. Bioaugmentation was achieved by delivering MC-100 culture under pressure through an open-ended Geoprobe Systems core barrel and group pump assembly. A total of approximately 6000L of MC-100 solution was delivered in 20L injections spaced every 0.3 m vertically and horizontally across the 6 m transect of the test plot.

Following injection of the MC-100 culture, MTBE concentrations in the bioaugmentation test plot decreased significantly after 30 days, and concentrations continued to decrease or remain low (< 0.01 mg/L) through 261 days after seeding. In the biostimulation test plot, MTBE concentrations decreased to 0.01 to 0.1 mg/L after a lag period of 173 to 230 days. The authors reported that TBA appeared to emanate untreated or partially treated from the biostimulation plot. Based on these collective observations, Salanitro et al. (2000) concluded that bioaugmentation with MC-100 may be preferred in cases even if indigenous MTBE-degraders are present at a site. It should be noted that while bioaugmentation appeared to achieve MTBE biodegradation much more quickly, the demonstration did not allow a determination of whether MTBE biodegradation rates in the bioaugmentation plot were significantly faster than biodegradation rates in the biostimulation plot (after the onset of biodegradation).

Wilson et al. (2002) identified one key limitation that reduces the strength of the conclusions of Salanitro et al. (2000). That limitation is that the work of Salanitro et al. (2000) did not account for the potential reduction in permeability in the test plot resulting from the entrainment of oxygen gas in the aquifer pore space. This reduction in permeability might have lead to reduced groundwater flow through the treatment zones, resulting in partial bypass of contaminated groundwater around the test plots. This possibility imparts uncertainty to the meaning of the MTBE time series data for the Salanitro et al. (2000) field demonstration. Wilson et al. (2002) concluded that given that there was some uncertainty regarding the flow field through the test plots, the Salanitro et al. (2000) field data did not yield reliable estimates of the rate of MTBE biodegradation in the biostimulation and bioaugmentation test plots.

2.2.8 Kelly Air Force Base, San Antonio, Texas

A field demonstration of bioaugmentation for treating dissolved-phase chlorinated solvents in groundwater at Kelly Air Force Base in San Antonio, Texas was demonstrated by Major et al. 2002. Prior to the initiation of the demonstration, the site groundwater contained about 1 mg/L of PCE and lower amounts of TCE and c-DCE. The existing site data suggested that the dechlorination that was occurring intrinsically had stalled at c-DCE. The demonstration used the dechlorinating culture KB-1, a natural anaerobic culture that was enriched from soil and groundwater obtained from a TCE-contaminated site in Ontario (Duhamel et al. 2002). KB-1 contains phylogenetic relatives of *Dehalococcoides ethenogenes*, and is capable of rapidly dechlorinating PCE and TCE to ethene.

Laboratory microcosm studies using groundwater and aquifer material from the pilot test area (PTA) were performed prior to the field demonstration in order to determine if the native microorganisms could be stimulated to achieve effective dechlorination. The microcosms were also used to test the efficacy of bioaugmentation. Seven treatments were evaluated, including sterile control, intrinsic control, lactate-amended, methanol amended, and three methanol-amended and bioaugmented. The intrinsic control microcosms were not amended with any exogenous electron donor. TCE was used as the primary test compound. There was no significant loss of TCE in sterile or intrinsic control microcosms and no formation of transformation products after 120 days of incubation. Stoichiometric conversion of TCE and c-DCE was observed in the lactate and methanol-amended microcosms, and neither treatment achieved dechlorination past c-DCE. In all of the microcosms treated with KB-1 and methanol, all of the TCE was converted stoichiometrically to ethene. 16S rDNA-based PCR methods were used to screen groundwater and aquifer sediment from the PTA for the presence of *Dehalococcoides* microorganisms. *Dehalococcoides* sequences were not detected in any of the six samples tested. These data suggested that intrinsic dechlorination at the site was limited, in part, by an absence of *Dehalococcoides*.

The field demonstration employed a closed-loop groundwater recirculation system in a shallow, unconsolidated sand and gravel aquifer. Groundwater was extracted from series of three downgradient extraction wells, blended, treated with various additives, and injected into a single well located at the upgradient edge of the PTA. The injection well was located approximately 9.1 meters away from the middle extraction well, and the distance between each of the

extraction wells was approximately 0.9 meters. A series of performance monitoring wells were installed along the groundwater flowpath centerline between the injection well and middle extraction well. All wells were screened across the saturated aquifer thickness (approximately 3.1 meters). Two bromide tracer tests were conducted at the beginning to optimize groundwater capture and to estimate flow velocities within the PTA. A numerical flow model was calibrated with the bromide data, and used to identify optimal pumping rates to improve groundwater capture within the recirculation cell.

From the modeling analyses, a total flow rate of 5.7 L/min was chosen. Assuming a porosity of 0.3, the approximate volume of water in the PTA was 64,000L. The estimated average linear flow velocity was 14.3 m/day. Based on the time to recover the mass of bromide injected, the time to capture and recirculate one pore volume approximately 7.8 days.

The pilot test operation consisted of three general phases. In the first phase, groundwater was recirculated for 89 days to equilibrate the system and to conduct the bromide tracer test. In the second phase (day 90 until day 175), methanol and acetate were added as electron donors at a concentration of 3.6mM each. The objective of the second phase was to induce anaerobic conditions in the test plot, and to stimulate reductive dechlorination by the indigenous bacteria. The third phase (bioaugmentation) was initiated on day 176 and ended on day 319 (the end of the demonstration). Thirteen (13) liters of KB-1 culture (> 10^6 cells/mL) were delivered into the injection well on day 176. The culture was delivered via pressure displacement from two 8L stainless reactor tanks that were used to grow and ship the culture. Groundwater recirculation and delivery of electron donor resumed 24 hours after the KB-1 culture was introduced into the test cell, and continued until the end of the test ended on day 319.

Performance monitoring from the pilot showed that in the presence of methanol and acetate, the indigenous bacteria could be stimulated to dechlorinate PCE to c-DCE. However, no dechlorination past c-DCE was observed. After the addition of the KB-1 culture, the chlorinated ethenes were completely degraded to below drinking water standards within 200 days. Once biodegradation was established, calculated half-lives for degradation were on the order of minutes to hours. The biodegradation rates observed in the field were > 1 order of magnitude faster than rates observed in laboratory treatability studies. The authors hypothesized that the field rates were faster because the pilot used a continuously flowing, continuously fed system that supported biofilm growth, while the laboratory study used a more static, slower growing system.

16S rDNA-based PCR methods were used to monitor the migration and growth of KB-1 culture after injection. Although KB-1 migration was retarded significantly relative to groundwater flow, molecular monitoring showed that culture had completely colonized the 9.1 meter-long aquifer test plot within 115 days after the one-time injection of KB-1. No clogging of the injection well was observed.

In addition to the PTA, two biostimulation control plots were installed and operated in the same manner as the PTA. Both control plots were amended with the same concentrations of electron donor as the PTA, but were never amended with KB-1. In these control plots dechlorination stalled at c-DCE, with no vinyl chloride observed during 216 days of operation. PCR analysis confirmed that *Dehalococcoides* was not indigenous in the control test plots.

This field demonstration can be considered a successful application of bioaugmentation technology for remediation of chlorinated solvents in groundwater. Numerous lines of evidence were presented that confirm the role of bioaugmentation in catalyzing treatment, including: (1) incomplete dechlorination in electron-donor amended laboratory microcosms; (2) incomplete dechlorination after 216 days in biostimulated control plots; (3) absence of *Dehalococcoides* DNA sequences in the control plots and in the PTA before addition of KB-1; (4) stoichiometric conversion of PCE to ethene in the PTA after bioaugmentation with KB-1; (5) detection of specific DNA sequences unique to KB-1 in the PTA after bioaugmentation, and (6) increased abundance of KB-1 DNA sequences over time. Collectively, these data indicate that *Dehalococcoides* colonized the PTA as a result of KB-1 injection, and grew via the respiration with chloroethenes as the electron acceptor and methanol and acetate as the electron donor.

2.2.8 Bachman Road Residential Wells Site, Lake Huron, Michigan

Lendvay et al. (2001) conducted a bioaugmentation pilot study at the Bachman Road Residential Wells Site. Like the demonstrations performed by Ellis et al. (2000) and Major et al. (2001; 2002), the pilot study at the Bachman Road site involved the injection of a culture containing phylogenetic relatives of *Dehalococcoides ethenogenes* and electron donor to stimulate complete reductive dechlorination of chlorinated ethenes in groundwater. The groundwater plume at the site is contaminated predominantly with PCE, and the flows into Lake Huron. Field data collected prior to the pilot study indicated that some dechlorination was occurring naturally, as evidenced by the accumulation of c-DCE and VC. Bioaugmentation was evaluated because the rate of natural dechlorination was

insufficient to prevent the plume from discharging into Lake Huron. An enrichment inoculum (the Bachman Road Culture) containing *Desulfuromonas* and *Dehalococcoides* derived from soil and groundwater samples from the site was used for bioaugmentation.

Like Major et al. (2001; 2002), this study also compared chloroethene treatment performance in a bioaugmented plot and a control plot. No electron donor was delivered to the control plot, and both plots employed closed-loop, recirculatory hydraulic control systems. Each plot used one injection well, two extraction wells, and a variety of multi-level monitoring points located upgradient, downgradient, transgradient, and between the injection and extraction wells. The distance between the injection well and the plane created by the two extraction wells was 3.05 m in both the bioaugmented plot and the control plot. The each plot was oriented normal to groundwater flow.

Beginning one month prior to inoculation, the bioaugmented plot was fed 0.1 mM lactate and nutrients (nitrogen and phosphorus) to create uniform anaerobic conditions in the test plot. After anaerobic conditions were established, approximately 210 L of microbial suspension (10^8 cells/mL) was injected into the aquifer, followed by continuous injection of 0.1 mM lactate and nutrients. Complete dechlorination of the chloroethenes was achieved within the bioaugmented plot 50 days after injection of the Bachman Road culture. Over the same period, c-DCE concentrations in the control plot increased approximately by a factor of 2, indicating active dechlorination of PCE and TCE. The authors suggested that co-occurring fuel hydrocarbon contamination in the groundwater served as the electron donor that supported dechlorination in the control plot.

PCR analysis and 16S rDNA information were used to assess the microbiology of the bioaugmentation and control plots. *Dehalococcoides* was detected in the control plot prior to inoculation, and the spatial extent of *Dehalococcoides* detection in the control plot increased the two month monitoring period following inoculation. In the bioaugmented plot, *Dehalococcoides* was not detected in the extraction well on day 0, but was detected at the extraction well on day 35 and day 69.

The results of the Bachman Road pilot test, as reported in Lendvay et al. (2001) demonstrate that complete dechlorination of chloroethenes could be achieved rapidly in a recirculatory flow system that provided lactate, nutrients, and the Bachman Road culture to the subsurface. Since the control plot was not amended with electron donor, however, the observed stimulative effect in the bioaugmented plot cannot be conclusively attributed to the addition of the Bachman Road culture. Although experience at other bioaugmentation sites would suggest that bioaugmentation was the primary cause of the rapid dechlorination rates observed at Bachman Road, the presence of indigenous *Dehalococcoides* and *Desulforomonas* populations at the site prevented assessment of the relative importance of electron donor addition and bioaugmentation.

2.2.10 Aerojet General Corporation, Sacramento, California

A field demonstration was initiated to assess TCE dechlorination in a deep aquifer at the Aerojet Superfund site in California (Cox et al., 2000; 2002). Previous laboratory microcosm studies for the Aerojet site had shown that TCE dechlorination consistently stalled at c-DCE, unless bioaugmented with dehalorespiring bacteria. The addition of lactate alone to the pilot test area (PTA) groundwater failed to promote significant TCE dechlorination past cis-1,2-DCE (VC and ethene were not produced). Bioaugmentation of the PTA with KB-1 immediately accelerated the rate of TCE and c-DCE dechlorination, and VC and ethene production from cis-1,2-DCE were observed within 8 days following bioaugmentation. Within 125 days, the concentrations of TCE (starting from 2 mg/L), c-DCE, 1,1-DCE and VC were below respective MCLs in the PTA. Molecular characterization techniques (16S rRNA screening using PCR) were used to evaluate the presence of *Dehalococcoides*: i) prior to bioaugmentation, to assess the effects of electron donor addition alone; and ii) following bioaugmentation to track the success of KB-1 addition, and to assess its transport and survival in the PTA groundwater. Initial sample analyses were negative, suggesting that *Dehalococcoides* was not present in the PTA groundwater. A few days after bioaugmentation, a strong signal representative of the *Dehalococcoides* strain in KB-1 was detected in the PTA well where KB-1 was introduced to the aquifer. A final sample round for *Dehalococcoides* was collected 75 days after bioaugmentation, and all wells in the PTA, to a distance of 50 feet from the point of introduction, indicated moderate to strong DHE signal suggesting transport of KB-1 through the PTA.

2.3 Summary of Recent Achievements

The understanding and application of in situ bioaugmentation technologies, for certain types of contaminants, has grown substantially in the past few years. Field demonstrations at Dover AFB and Kelly AFB provided strong evidence that bioaugmentation was required to achieve complete biological dechlorination of TCE in groundwater plumes at those sites. Although it has been proposed that bioaugmentation is seldom necessary for groundwater remediation (Suthersan, 2001), a growing number of comprehensive field demonstrations now indicate that bioaugmentation is often a necessary pre-requisite for achieving effective biological treatment of chlorinated solvents in groundwater.

The increasingly common use of 16S rDNA-based PCR methods have allowed microbiologists to identify and characterize microbial strains that mediate key biodegradation reactions (e.g., *Dehalococcoides ethenogenes*, *Hydrogenophaga flava*, and *Pseudomonas stutzeri).* For certain microbial cultures, the development and application of these DNA-fingerprinting techniques has provided a highly sensitive means for monitoring microbial growth and transport during bioaugmentation. The utility of molecular monitoring for field-scale bioaugmentation applications has been established in trials at Dover AFB, Kelly AFB, Port Hueneme, the Schoolcraft Test Site, the Bachman Road Residential Wells site, and a variety of other sites. In addition, due to their sensitivity for detecting low bacterial concentrations, molecular monitor techniques may eventually replace biotreatability microcosm studies for certain types of applications.

Through the course of successful field demonstrations, practitioners now possess improved understanding of bioaugmentation design parameters, including hydraulic control requirements, biomass dosing requirements, and, in the case of chlorinated solvents, electron donor dosing requirements. Bioaugmentation has been successfully applied in the field for treatment of dissolved phase chloroethenes and chloromethanes using recirculatory groundwater/reagent delivery systems. Successful treatment of with bioaugmentation has been achieved over a variety of designs, including plume-perimeter biobarrier systems operated on pulsed delivery schedule, to continuously operated systems located at the plume interior. Research has shown that the efficiency of in situ bioaugmentation treatment for chloroethenes depends, in part, on selection of an electron donor and delivery approach that minimizes methane generation.

3.0 RESEARCH NEEDS

Despite the success of the field trials described above, many questions remain. Additional field experience, including well-planned and monitored applications, are needed to gather the data required to advance the understanding of the underlying factors that affect the transport, survivability and activity of exogenous microorganisms. Although a substantial amount of previous research has been devoted to these topics, it is evident that microbial transport and survival characteristics can vary significantly from specie to specie and culture to culture. Future research should focus on factors affecting transport, survival, and activity of strains that are currently perceived to offer the most promise for widespread use in bioaugmentation applications. In addition, the field of bioaugmentation research would benefit greatly from the development of a standardized approach for collecting data necessary to understand factors governing microbial transport and survival.

Bioaugmentation involves the addition of isolated and metabolically enhanced microorganisms into the subsurface. However, there are some key factors that must be taken into account for successful application. First, even when aquifer formations are permeable, they can often behave as very effective filters for removing particulates. This can sometimes lead to difficulty in distributing microorganisms throughout any appreciable aquifer volume relative to the size of most plumes. It is generally known that distribution of exogenous cells can be improved by reducing ionic strength, selecting for motile strains, or selecting for non-hydrophobic strains, but the adhesion and filtration characteristics for certain key bioaugmentation strains and cultures has not been characterized. Until these data are available, our ability to mathematically simulate bioaugmentation performance will be limited.

Second, for some site conditions, injection of exogenous cultures may expose them to an environment that is hostile compared to the well-controlled condition in the laboratory fermentors where they are grown. The indigenous microflora have already adapted to the in situ environment and are quick to respond to any stimuli that are introduced. The injected culture may be considered one of these stimuli and the indigenous microflora may outcompete them for essential growth factors, use the cells as a source of nutrients, or adversely affect the metabolic activity for which the injected cells were selected. The geochemistry within the aquifer may also adversely affect

survivability and metabolic activity of the injected culture. One example is high-salinity or high sulfate environments that are characteristic of brackish aquifers. All of these variables must be better understood in order to advance the bioaugmentation technology.

Most of the research on bioaugmentation has occurred in the laboratory. While these studies have proven invaluable for the successful applications seen to date, additional field research is needed to move forward. This is primarily due to the fact that many of the variables that will affect the delivery, survivability, and activity of injected cultures will be site specific. Some examples of site-specific conditions that can affect bioaugmentation performance and thus need better understanding include:

- how aquifer mechanics affects bioaugmentation design requirements;
- how reagent delivery design (passive vs. active delivery of electron donor; flow control; pulsed vs. continuous nutrient delivery) affects treatment rates;
- how selection of bioremediation nutrients (e.g., electron donor) affect bioaugmentation performance and groundwater quality;
- how aquifer geochemistry can affect microbial transport, culture survivability, and performance;
- how indigenous microbial populations compete with, and affect the survivability of, the injected culture;
- how immiscible contaminants (DNAPLs) and complex mixtures can affect technology performance;
- how environmental conditions (i.e., temperature, pH, water potential, etc.) affect bioaugmentation performance; and
- how supplemental remedies (i.e., chemical oxidation, zero valent iron) affect bioaugmentation performance.

The following sections provide a brief discussion of each of these parameters, including recommendations for addressing each during field application of bioaugmentation.

3.1 Aquifer Mechanics

In order to effectively design and apply bioaugmentation at any site, it is necessary to have a good understanding of the hydrogeology. This includes understanding the hydraulics of the aquifer and how it will respond to pumping and injection of fluids. In addition, changes in the aquifer mechanics as the remedial activity progresses need to be understood.

A complete characterization of the hydrogeologic and geologic conditions at the site should be performed before initiating a bioaugmentation project. High and low permeability stratigraphic layers within the aquifer (e.g., alternating layers of coarse- and fine-grained materials), should be identified to evaluate the potential groundwater flow patterns at the project site. The microbial culture needs to be introduced into the stratigraphic layer(s) containing the contaminant of concern, and the introduction of the culture to these layer(s) may be inhibited by the permeability of these layers. For example, if the contaminants are contained in a low-permeability layer near the top of the aquifer, and higher permeability layers are present beneath this contaminated zone, it would be important at this hypothetical site to screen the injection wells appropriately (within the low permeability layer) to introduce the culture into the contaminated layer. If the injection wells are screened across both the low- and high-permeability layers, the culture will not move to the low-permeability zone and the treatment may not be effective. Also, the low permeability layers at the site need to be identified to determine the extent of the contamination and where DNAPL pools may be present.

Initial bioaugmentation project activities should include lithologic and hydraulic characterization of the project site. Coring can provide information on stratigraphic layering and should be included. Pumping tests should be performed at the actual injection location to determine the hydraulic conductivity. The natural hydraulic gradient and groundwater flow velocities also should be determined. These data will aid in determining the effects to the natural flow patterns when the culture, nutrients, or electron donating/accepting substrates are injected into the subsurface. If it is determined that a recirculation or extraction/injection style system is required for adequate distribution of the culture or amendments, this information will help determine proper pumping rates from and to the delivery wells.

Changes in the aquifer characteristics after the addition of microbial cultures, nutrients, and electron donating/accepting substrates are added to the aquifer should also be determined. The addition of the nutrients and electron donors/acceptors or microbial growth will likely change the geochemistry and permeability of the aquifer. Changes in groundwater geochemistry may result in the precipitation of minerals and resultant changes in the hydraulic conductivity at the site.

3.2 Reagent Delivery Design

As with any in situ aquifer restoration technology, direct contact between the contaminant and the remedial reagent(s) is critical for success. In the case of bioaugmentation, contact between the microorganisms and essential growth factors, is very important. For bioaugmentation to be effective, the added microorganisms must be brought into direct contact with the contaminant. The limitations of microbial transport suggest that it may be desirable to inject the culture and bring the contaminant to the cells. This would favor in situ biological barrier or biofilter configurations, which are set up by injecting the culture into a designated volume of the aquifer to establish an active zone across which the contaminant is either pumped or allowed to pass through with the flow of groundwater. Designing a system that provides intimate contact requires detailed aquifer testing and evaluation.

There is a range of bioaugmentation system designs (passive, semi-passive, fully active) that control delivery/flow, chemistry, and microbiology to varying degrees. If the site chemistry, microbial, and flow regime are not ideal, engineering can overcome these conditions to some extent. In general, the most rapid and effective treatment is achieved with fully engineered systems. The most successful bioaugmentation trials reported in the literature have involved groundwater recirculation, with some systems operated continuously and others operated on a pulsed schedule. Passive bioremediation systems that rely of natural flow gradients to deliver reagents to contaminants (e.g., one-time injection of low-solubility electron donors into biobarriers at chlorinated solvent sites) have gained popularity because of their relatively low capital cost; however the treatment performance of these systems can be significantly different than active bioremediation systems. Proximal to source areas, for example, passive bioremediation systems often fail to destroy contaminants effectively because the rate of bioremediation agent delivery is too slow to meet biodegradation requirements for the contaminant flux emanating from the source area. Technical guidance is needed to provide practitioners with appropriate tools and analyses for developing effective bioremediation/bioaugmentation designs.

3.3 Selection of Bioremediation Nutrients

With regard to enhanced bioremediation/bioaugmentation systems for treatment of chlorinated solvents, there are a variety of compounds and products that are used to induce anaerobic conditions and provide reducing equivalents to support biological reductive dechlorination. Compounds that are commonly used as electron donors include lactate-based polymers (Hydrogen Release Compound (HRC)), slow-release oil based compounds, soluble compounds such as sugars (molasses), food grade acids (acetate or lactate), and alcohols (methanol or ethanol). Some practitioners treat different types of electron donors interchangeably, and view them as essentially equivalent in terms of bioremediation performance. However, a growing body of data indicates the choice of electron donor compound can significantly affect treatment rates and consequent water quality during bioaugmentation applications. Some electron donors are more prone to enhance growth of microorganisms (e.g., methanogens or sulfate-reducing bacteria) that may outcompete introduced cultures (e.g., *Dehalococcoides ethenogenes*).

Some electron donors, by design, are applied in amounts that greatly exceed the stoichiometric demand posed by the chlorinated contaminants in the system. Consequently, injection of certain electron donors into the subsurface (e.g., vegetable oil, molasses, etc.) can often result in excess hydrogen production, which, in turn leads to excess methane production. Other undesirable side effects of this approach include elevated dissolved organic carbon, mobilization of metals, and sulfide production. Some researchers have questioned the benefit of replacing one class of contaminants (chlorinated solvents) with another class of contaminants (BOD, TDS, CH_4, H_2S, and metals). Research has shown that methane production, sulfide production, and mobilization of metals can be minimized (and, in some cases, eliminated) by adding only enough electron donor to meet the stoichiometric demand posed by the chlorinated constituents and other oxidized species in the plume (e.g., oxygen, nitrate).

Additional research is necessary to evaluate how varying the electron donor composition will affect performance of exogenous cultures. This research would have the greatest benefits for cultures that have been demonstrated to have

value for widespread application. In effect, this research would provide information on the operational ranges of the cultures evaluated. Findings from this research could be integrated into guidance regarding design of bioaugmentation systems.

3.4 Microbial Transport, Culture Survival, and Culture Activity

Microbial transport, culture survival, and culture activity are all interrelated, and bioaugmentation performance is dependent on the interaction of all three phenomena.

In order to be effective at field scale, it must be possible to distribute the culture across a sufficient volume of the aquifer to achieve the level of contaminant reduction required. This is due to the relationship between the number of microbes and kinetics of degradation, which is true for any configuration of application (i.e., biofilter, biobarrier, etc.). Microbial transport describes the movement of microorganisms in the aquifer. The process has been investigated in laboratory studies as discussed above, and the results have shown that the ability to deliver and distribute microorganisms into aquifers is affected by many variables. Some of these variables can be controlled in the field; others are logistically impossible to manipulate. While some of the past research has been more basic, the results have contributed to understanding the process. Future laboratory research should be more applicable to recognized field problems.

Culture survivability has been one of the most questioned aspects of bioaugmentation. This issue was raised when microorganisms were added at random with little regard given to their source, inoculum development procedures, metabolic capabilities, and/or growth requirements. The few attempts that were made to investigate culture survivability failed when it was not possible to isolate the added microorganisms from the soils after short time periods. Providers of bioaugmentation products ignored this problem and suggest that adding more culture is the solution.

Often, survivability is addressed during bench-scale or microcosm studies where the environmental conditions (chemical and biological) in the test apparatus can be easily controlled. Research using laboratory microcosms is valuable for screening purposes but extrapolation to field conditions is often difficult because of the many unknowns encountered in situ, and because the environment within the aquifer is not easily controlled. The conditions established during the microcosm tests need to be investigated to determine the feasibility of establishing the same conditions in the field. The advancement of bioaugmentation would benefit from development of a standardized microcosm approach that would provide the data needed to assess the potential for survivability along with other screening activities.

DNA fingerprinting techniques may overcome many of the limitations inherent in more traditional methods for assessing culture transport and survival. 16S rDNA sequences have been mapped for certain species that mediate beneficial degradative reactions (e.g., PM1, *Desulfuromonas*, *Pseudomonas stutzeri* strain KC). Research has shown that the unique genetic signatures of certain exogenous cultures and isolates can be used to assess the need for and performance of bioaugmentation. Additional research is needed to further characterize the genetic composition (and phylogeny) of mixed cultures to elucidate the relative roles and importance of interacting strains. As new cultures are developed for bioaugmentation purposes, use of 16S rDNA sequencing and PCR analysis should become a routine step in culture characterization.

Although 16S rDNA-based PCR techniques are commonly used to monitor transport and survival of bioaugmentation cultures, standardized protocols are needed to provide guidance applying this technology. In addition, more development of PCR primer, probe, and array-technologies is needed in order to develop simple and accurate tests for species monitoring in environmental samples.

Data from several investigations suggests that the application of 16S rDNA-based PCR methods may be a more sensitive measure than microcosm studies for detecting biodegradation potential. Research is needed to provide a better understanding of the relative costs and benefits of determining biodegradation potential with molecular methods vs. microcosm studies.

Microbial transport and survivability are extremely important variables with direct implications for bioaugmentation success. Equally important is the ability of the culture to retain the desired metabolic capacity for a prolonged

period of time. To date, much of the research related to culture survivability has been limited to recovery of cells that grow on specific nutrient broth or agar plates. Simply recovering an organism from groundwater samples collected at distances from an injection point and growing them on nutrient-rich and selective media does not demonstrate successful bioaugmentation. Growth in these media alone does not provide data to evaluate biocatalytic potential, which is necessary for successful bioaugmentation.

3.5 Species Competition

Laboratory research has shown that survival and proliferation of exogenous cultures depends, in part, on competition by indigenous cultures. For chlorinated solvents in anaerobic systems, the relative success of indigenous cultures can depend, in part, on the type and concentration of electron donor that is provided to stimulate dechlorination. For example, methanogenic bacteria have been shown to outcompete certain dechlorinating cultures, depending on the type and concentration of electron donor. Certain dechlorinating cultures have also been shown to outcompete methanogens in the presence of high chlorinated solvent concentrations. In shallow aquifers, protozoa grazing may limit or control proliferation of exogenous cultures. These relationships illustrate that the importance of species competition can vary from site to site, and spatially within a single site. Research is needed to elucidate the role of species competition as an operational parameter for bioaugmentation system design (e.g., what factors contribute to proliferation of sulfate-reducing bacteria and methanogens over Bachman Road culture). Since exogenous cultures often possess unique characteristics with regard to species competition, research should consider how observed competition behavior may vary from culture to culture.

3.6 DNAPLs and Complex Mixtures

For certain types of contaminants, bioaugmentation is increasingly being recognized as a viable technology for containing and remediating dissolved phase contamination. While laboratory studies indicate that bioaugmentation offers promise for treatment of immiscible phase contaminants, the feasibility using bioaugmentation to remediate source areas in the field has not been demonstrated yet. ESTCP and the National Aeronautic Space Administration (NASA) are currently funding chlorinated solvent DNAPL bioaugmentation projects at Dover AFB and Kennedy Space Center, respectively. These and other field demonstrations will be required before the feasibility of using bioaugmentation to remediate chlorinated solvent DNAPL sources can be determined. The Dover AFB and NASA trials are being performed with the KB-1 culture. Additional research is needed to determine whether other cultures (e.g, strain KC or Pinellas culture) are capable of (1) surviving proximal to DNAPLs; (2) biodegrading chlorinated solvents at the water/DNAPL interface; and (3) significantly increasing the rate of DNAPL dissolution. If bioaugmentation is found to achieve significant treatment of DNAPL source areas in the field, this finding will have substantial implications for the value and use of bioaugmentation technology. Research in this area should also examine concentration toxicity, the effect of concentration of biodegradation rates, and determine biodegradation kinetic parameters (e.g., utilization constants, half-saturation constants, etc.) for specific cultures/isolates.

The value of bioaugmentation technology will also be improved cultures promise for field application are evaluated to determine their treatment performance in mixed substrate systems. Many laboratory and field trials that have observed successful bioaugmentation performance have involved single-substrate systems, or systems with only a few target contaminants. At many contaminated sites, particularly sites contaminated with chlorinated solvents, a diversity of organic compounds are present that may impact bioaugmentation performance. Halogenated ethenes, ethanes, methanes, and propanes may be present as mixed species in a plume, and research has shown that the presence of certain halogenated compounds may inhibit biodegradation of other halogenated compounds for certain exogenous cultures. Additional research is needed to provide an improved understanding of how multi-substrate systems can affect bioaugmentation performance for individual cultures.

One approach to addressing sites contaminated with multiple halogenated compounds is to utilize multiple exogenous cultures for bioaugmentation applications. For example, at sites where 1,1,1-TCA and chloroethenes are present as mixed contaminants, one effective bioremediation approach may be to deliver a bioaugmentation mixture that consists of known chloroethane degraders (e.g., TCA1) and known chloroethene degraders (e.g., KB-1). This approach warrants consideration, given the widespread co-occurrence of chloroethane and chloroethene contamination in groundwater.

3.7 Environmental Conditions

A variety of exogenous cultures have been shown to biodegrade target contaminants rapidly in highly controlled laboratory reactors, under optimal pH, temperature, and nutrient conditions. Many of these same cultures have performed reasonably well in field trials at sites where groundwater conditions were amenable to microbial growth and survival. However, as bioaugmentation continues to attract interest as a viable remediation technology, there are numerous sites where the technology might be implemented that have aquifer chemistry that is less than ideal for microbial proliferation. pH extremes (< 5 or > 9), high dissolved metals, or high ionic strength (high salinity) are all factors that can reduce the performance of exogenous cultures to varying degrees.

The effects of groundwater chemistry extremes can be overcome to some extent through engineering design. For example, in recirculatory flow systems, pH buffers and nutrients (nitrogen and phosphorus) can often be introduced and uniformly distributed within the treatment zone to achieve and maintain optimum environmental conditions. However, it is often difficult to control environmental conditions at sites where passive, or semi-passive reagent delivery systems are employed. In addition, even at sites where active reagent delivery systems are used, environmental conditions may become inhibiting at some distance from the active treatment zone. Consequently, it is important to understand how performance of exogenous cultures may vary as a function of pH, ionic strength, and other factors (e.g., specific metals, anions, etc.). For exogenous cultures that offer viable potential for widespread application, research is necessary to determine chemistry operational ranges. This data is necessary to ensure that bioaugmentation cultures are not applied in aquifers where chemical data indicates *a priori* that culture performance will be poor.

3.8 Performance Monitoring

The advancement of bioaugmentation would benefit from the development of technical guidance regarding requirements for monitoring performance of bioaugmentation systems. One reason why bioaugmentation technology has not developed more quickly is that field trials evaluating the technology have not employed test designs that allow a true measure of technology performance. In some cases it has not been possible to distinguish the effect of the exogenous cultures from the effects of indigenous cultures, nutrient addition, and/or dilution. Proper evaluation of bioaugmentation performance requires experimental controls to track stimulation of indigenous cultures, as well as changes in contaminant concentration due to dilution (or obstruction) caused by injection of reagents. In addition, bioaugmentation demonstrations and pilot tests require monitoring programs with sufficient sampling points and frequencies to provide a statistically meaningful measure of spatial and temporal contaminant concentration trends.

Molecular monitoring provides an excellent tool for monitoring migration and growth of injected cultures during bioaugmentation. As such, molecular methods can serve as a valuable component of any bioaugmentation performance monitoring program. In addition to monitoring the injected culture, performance monitoring must also track distribution of added nutrients and contaminant destruction (including transformation daughter products). Proper mass balance analysis (e.g., developing a careful budget of system inputs and outputs) can be a key determinant of whether a bioaugmentation test is considered a success. Technical guidance is needed regarding design of monitoring programs that allow analysis of contaminant and nutrient mass flux and mass balance.

3.9 Supplemental and Complementary Remedies

At many sites where enhanced in situ bioremediation technologies (including bioaugmentation) are applied, other remedies (e.g., chemical oxidation, co-solvent flushing, or thermally-enhanced extraction) have been implemented previously, or are implemented concurrent with bioremediation. In situ bioremediation and/or bioaugmentation is often chosen as a "polishing step" to destroy source area residual after other source area remedies have been completed. Research is needed to provide a better understanding of how these other source area remedies impact bioaugmentation performance.

In situ chemical oxidation (ISCO) involves injecting Fenton's Reagent, permanganate, or persulfate to oxidatively destroy organic contaminants. For treatment of chlorinated solvent source areas, ISCO poses obvious problems for bioremediation strategies that involve anaerobic reductive dechlorination (ISCO and anaerobic reductive dechlorination require opposite redox conditions). It has been proposed that certain ISCO applications tend to dramatically reduce and or/inhibit biodegradation activity by indigenous microflora. Bioaugmentation is an

attractive option for inoculating source areas after ISCO applications have been completed. However, certain ISCO remedies (e.g., permanganate) may create elevated redox conditions that could inhibit anaerobic bioaugmentation remedies for many months to years after the ISCO treatment was completed (e.g., MnO_2, a product of permanganate oxidation, may accumulate and poise the redox at a level that is too high to support biological reductive dechlorination). SERDP is presently funding research investigating the suitability of sequencing ISCO and anaerobic bioaugmentation remedies for chlorinated solvent source area treatment. Continued research by ESTCP/SERDP and other organizations is needed to determine the parameters for successfully implementing sequenced ISCO and bioaugmentation.

Solvent or co-solvent flushing emerged in the 1990s as a potentially effective technology for mobilizing and removing DNAPL residual from source areas. Co-solvents including ethanol and methanol, often added to achieve aqueous concentrations $\geq$ 10% solvent, have successfully mobilized chlorinated solvent DNAPLs at a variety of sites. Given that ethanol and methanol can also serve as preferred electron donors for bioaugmentation applications involving reductive dechlorination, research is needed to investigate the feasibility of remediating DNAPL source areas using co-solvent flushing followed by bioaugmentation. A critical issue in this regard is toxicity of high concentrations of alcohol to bioaugmentation cultures. Working with well characterized and promising cultures for chlorinated solvent bioaugmentation (e.g., TCA1, KB-1, Bachman Road culture), studies should be performed to identify toxic thresholds for alcohols. Research on this topic should consider potential deleterious side effects of biodegradation of high concentrations of alcohols (e.g., excessive methane formation).

At sites contaminated with chlorinated solvents and other relatively oxidized contaminants (e.g., nitroaromatics, hexavalent chromium, etc.), zero valent iron (ZVI) is often delivered in permeable reactive barriers (PRBs) to intercept and treat migrating contaminant plumes. Bacteria can colonize ZVI PRBs naturally, and bioaugmentation with exogenous cultures may further increase the extent of biodegradation in ZVI PRBs. Research has shown that augmenting ZVI treatment systems with anaerobic bacteria can increase the rate and extent of transformation of some common types of contaminants. Bioaugmentation may be able to improve the long-term performance of ZVI PRBs by removing iron oxides and hydrogen gas (H_2) bubbles that can reduce the reactivity of the PRB. Certain anaerobic cultures can utilize the H_2 generated by ZVI to biologically reduce and destroy the same contaminants being treated abiotically by ZVI. Research is needed to elucidate the role of bacteria in ZVI PRBs, and to quantify the benefits of bioaugmenting ZVI PRBs. SERDP and ESTCP are currently research projects investigating the benefits of and design parameters for augmenting ZVI PRBs with bacteria. Future research should also examine how different exogenous cultures (e.g., TCA1, strain KC, KB-1) effect short-term and long-term treatment performance for specific groups of contaminants in ZVI PRBs.

3.10 Conclusion

Overall, the advancement and acceptance of bioaugmentation will continue only after increasing numbers of successful field demonstrations. The recent advances made were a result of well-thought-out and planned approaches both in the laboratory and field. The technology would undoubtedly benefit from a standardized approach such as those described in the protocols that have been and currently are being developed under ESTCP. Having such an approach would ensure that the data needed to evaluate the promise of the technology would be collected, which in turn would lead to an effective and cost-efficient application procedure for screening, designing, and applying bioaugmentation.

4.0 REFERENCES

Bourquin, A.W., D.C. Mosteller, R.L. Olsen, M.J. Smith, and K.F. Reardon. 1997. "Aerobic Bioremediation of TCE-Contaminated Groundwater: Bioaugmentation with *Burkholderia cepacia* PR1301." In B.C. Alleman and A. Leeson (Eds), *In Situ and On-Site Bioremediation: Volume 4.* Battelle Press, Columbus, OH. p. 513-518.

Burlage, R.S., A.V. Palumbo, and J. McCarthy. 1995. "Signal Quantification of Bacteria for a Groundwater Transport Experiment." In R.E. Hinchee, J. Fredrickson, and B.C. Alleman (Eds), *Bioaugmentation for Site Remediation*. Battelle Press, Columbus, OH. p. 139-148.

CDM. See Camp Dresser and McKee.

Camesano, T.A., and B.E. Logan. 1998. "Influence of Fluid Velocity and Cell Concentration on the Transport of Motile and Nonmotile Bacteria in Porous Media." *Environ. Sci. Technol.* **32**(11):1699-1708.

Camp Dresser and McKee. 1998. "Bioaugmentation Pilot Study: New Microbe Degrades Chlorinated Solvents." http://www.cdm.com/Svcs/haswaste/biorem.htm.

Carr, C.S., S. Garg, and J.B. Hughes. "Effect of Dechlorinating Bacteria on the Longevity and Composition of PCE-Containing Nonaqueous Phase Liquids Under Equilibrium Dissolution Conditions." *Environ. Sci. Technol.* 34(6): 1088-1094.

Chang, P.R., P.J. Zeeb, C.R. Elder, R.B. Leary, G.A. Demers, D.W. Major, and D.A. Wanty. 2002. "The Evolving Microbiology of an Enhanced Bioremediation Pilot Test Performed in Fractured Bedrock. " Presented at the Battelle Third International Conference on Remediation of Chlorinated and Recalcitrant Compounds. Monterey California, May 20-23.

Cope, N. and J.B. Hughes. 2001. "Biologically-Enhanced Removal of PCE from NAPL Source Zones." *Environ. Sci. Technol.* 35: 2014-2021.

Cox, E.E., E.A. Edwards, S.L. Neville, G. Swanick, and D.W. Major. 2000. "Accelerated Bioremediation of Perchlorate and Trichloroethene in Groundwater." In: Remediation of Chlorinated and Recalcitrant Compounds, Vol. 2, Battelle Press.

Deeb, R.A., H.-Y. Hu, J.R. Hanson, K.M. Scow, and L. Alvarez-Cohen. 2001. "Substrate Interactions in BTEX and MTBE Mixtures by an MTBE-Degrading Culture." *Environ. Sci. Technol.* 35(2):312-317.

DeFlaun, M.F., S.R. Oppenheimer, S. Streger, C.W. Condee, and M. Fletcher. 1999. "Alterations in Adhesion, Transport, and Membrane Characteristics in an Adhesion-Deficient Pseudomonad." *Appl. Environ. Microbiol.* 56:112-119.

Duba, A.G., K.J. Jackson, M.C. Jovanovich, R.B. Knapp, and R.T. Taylor. 1996. "TCE Remediation Using In Situ Resting-State Bioaugmentation." *Environ. Sci. Technol.* **30**(6):1982-1989.

Duhamel, M., S.D. Wehr, L.Yu, H. Rizvi, D. Seepersad, S. Dworatzek, E.E. Cox, and E.A. Edwards. 2002. "Comparison of Anaerobic Dechlorinating Enrichment Cultures Maintained on Tetrachloroethene, Trichloroethene, *cis*-Dichloroethene, and Vinyl Chloride." *Wat. Res.* 36:4193-4202.

Duston, K.L., M.R. Wiesner, and C.H. Ward. 1997. "Bacterial Attachment and Transport through Porous Media: The effects of Bacterial Cell Characteristics." In B.C. Alleman and A. Leeson (Eds), *In Situ and On-Site Bioremediation: Volume 4*. Battelle Press, Columbus, OH. p. 565.

Dybas, M.J., G.M. Tatara, W.F. Knoll, T.J. Mayotte, and C.S. Criddle. 1995. "Niche Adjustment for Bioaugmentation with *Pseudomonas* sp. Strain KC." In R.E. Hinchee, J. Fredrickson, and B.C. Alleman (Eds), *Bioaugmentation for Site Remediation*. Battelle Press, Columbus, OH. p. 77-84.

Dybas, M.J., M. Barcelona, S. Bezborodnikov, S. Davies, L. Forney, H. Heuer, O Kawka, T. Mayotte, L. Sepulveda-Torres, K. Smalla, M. Sneathen, J. Tiedje, T. Voice, D.C. Wiggert, M. Witt, and C. Criddle. 1998. "Pilot-Scale Evaluation of Bioaugmentation for In-Situ Remediation of a Carbon Tetrachloride-Contaminated Aquifer." *Environ. Sci. Technol.* **32**(22):3598-3611.

Dybas, M.J., D.W. Hyndman, R. Heine, J. Tiedje, K. Linning, D. Wiggert, T. Voice, X. Zhao, L. Dybas, and C.S. Criddle. 2002. "Development, Operation, and Long-Term Performance of a Full-Scale Biocurtain Utilizing Bioaugmentation." *Environ. Sci. Technol.* 36(16):3635-3644.

Ellis, D.E., E.J. Lutz, J.M. Odom, R.J. Buchanan, C.L. Bartlett, M.D. Lee, M.R. Harkness, and K.A. Deweerd. 2000. "Bioaugmentation for Accelerated In Situ Anaerobic Bioremediation." *Environ. Sci. Technol.* 34(11):2254-2260.

Fennell, D.E., J.M. Gossett, and S.H. Zinder. 1997. "Comparison of Butyric Acid, Ethanol, Lactic Acid, and Proponic Acid as Hydrogen Donors for the Reductive Dechlorination of Tetrachloroethene." *Environ. Sci. Technol.* 31(3):918-926.

Fennell, D.E., and J.M. Gossett. 1998. "Modeling the Production and Competition for Hydrogen in a Dechlorinating Culture." *Environ. Sci. Technol.* 32(16):2450-2460.

Fennell, D.E., A.B. Carroll, J.M. Gossett, and S.H. Zinder. 2001. "Assessment of Indigenous Reductive Dechlorinating Potential at a TCE-Contaminated Site Using Microcosms, Polymerase Chain Reaction Analysis, and Site Data." *Environ. Sci. Technol.* 35(9):1830-1839.

Gerritse, J., V. Renard, T.M. Pedro-Gomes, P.A. Lawson, M.D. Collins, and J.D. Gottschal. 1996. "*Desulfitobacterium* sp. Strain PCE1, an Anaerobic Bacterium that Can Grow by Reductive Dechlorination of Tetrachloroethene or *ortho*-Chlorinated Phenols." *Arch. Microbiol.* 165:132-140.

Hanson, J.R., C.E. Ackerman, and K.M. Scow. 1999. "Biodegradation of Methyl tert-Butyl Ether by a Bacterial Pure Culture."*Appl. Environ. Microbiol.* 65(11):4788-4792.

Harkness, M. R.; A.A. Bracco, M.J. Brennan Jr., K.A. DeWeerd, and J.L. Spivack. 1999. "Use of Bioaugmentation To Stimulate Complete Reductive Dechlorination of Trichloroethene in Dover Soil Columns." *Environ. Sci. Technol.; 33*(7): 1100-1109.

Harvey, R.W., and S.P. Garabedian. 1991. "Use of Colloid Filtration Theory in Modeling Movement of Bacteria Through a Contaminated Sandy Aquifer." *Environ. Sci. Technol.*, 25(1):178-185.

Hatzinger, P.B., K. McClay, S. Vainberg, M. Tugusheva, C.W. Condee, and R.J. Steffan. 2001. "Biodegradation of Methyl tert-Butyl Ether by a Pure Bacterial Culture." *Appl. Environ. Microbiol.* 67(12): 5601-5607.

Hendrickson, E.R., M.G. Starr, M.A. Elberson, J.A. Payne, E. Mack, H.-B. Huang, M. McMaster, and D.E. Ellis. 2001. "Using a Molecular Monitoring Approach to Monitor a Bioaugmentation Plot." In: Leeson, A., B.C. Alleman, P.J. Alvarez, and V.S. Magar (Eds.) *Bioaugmentation, Biobarriers, and Biogeochemistry*. Battelle Press, Columbus, OH. pp. 43-51.

Hendrickson, E.R, J.A. Payne, R.M. Young, M.G. Starr, M.P. Perry, J.A. Payne, and L.W. Buonamici. 2002. "Molecular Analysis of Dehalococcoides 16S ribosomal DNA from Chloroethene-Contaminated Sites Throughout North America and Europe. *Appl. Environ. Microbiol.* 68:485-495.

Holliger, C. D., D. Hahn, H. Harmsen, W. Ludwig, W. Schumacher, B. Tindall, F. Vazquez, N. Weiss, and A.J.B. Zehnder. 1998. "*Dehalobacter restrictus* gen. nov. and sp. nov., a Strictly Anaerobic Bacterium that Dechlorinates Tetra- and Trichloroethene in an Anaerobic Respiration." *Arch. Microbiol.* 169:313-321.

Hristova, K.R., C.M. Lutenegger, and K.M. Scow. 2001. "Detection and Quantification of Methyl tert-Butyl Ether-Degrading Strain PM1 by Real-Time Taqman PCR." *Appl. Environ. Microbiol.* 67(11):5154-5160.

Hughes, J.B., K.L. Dunston, and C.H. Ward. 2002. *Engineered Bioremediation*. Ground-Water Remediation Technologies Analysis Center Technology Evaluation Report TE-02-03. Pittsburgh, PA. http://www.gwrtac.org/pdf/bio_e.pdf

Huling, S.G., and J.W. Weaver, 1991. "Dense Nonaqueous Phase Liquids." Ground Water Issue. U.S. Environmental Protection Agency. EPA/540/4-91-002.

Hyndman, D. W., M. J. Dybas, L. Forney, R. Heine, T. Mayotte, M. S. Phanikumar, G. Tatara, J. Tiedje, T. Voice, R. Wallace, D. Wiggert, X. Zhao, and C. S. Criddle, 2000. "Hydraulic Characterization and Design of a Full-scale Biocurtain." *Ground Water* 38(3): 462-474.

Jennings, D.A., J.N. Petersen, R.S. Skeen, B.M. Peyton, B.S. Hooker, D.L. Johnstone, and D.R. Yonge. 1995. "An Experimental Study of Microbial Transport in Porous Media." In R.E. Hinchee, J. Fredrickson, and B.C. Alleman (Eds), *Bioaugmentation for Site Remediation*. Battelle Press, Columbus, OH. p. 97-103.

Krumholz, L.R., 1997. "Desulfuromonas chloroethenica sp. nov. Uses Tetrachloroethene and Trichloroethene as Electron Acceptors." *Int. J. Syst. Bacteriol.* 47:1262-1263.

Li, Q. and B.E. Logan. 1999. "Enhancing Bacterial Transport for Bioaugmentation of Aquifers using Low Ionic Strength and Surfactants." *Water Res.* **33**(4):1090-1100.

Lendvay, J., P. Adriens, M. Barcelona, C.L. Major, Jr., J. Tiedje, M. Dollhopf, F. Loffler, B. Fathepure, E. Petrovskis, M. Gebhard, G. Daniels, R. Hickey, R. Heine, and J. Shi. 2001. "Preventing Contaminant Discharge to Surface Waters: Plume Control with Bioaugmentation." In: Leeson, A., B.C. Alleman, P.J. Alvarez, and V.S. Magar (Eds.), *Bioaugmentation, Biobarriers, and Biogeochemistry*. Battelle Press, Columbus OH. pp. 19-26.

Loffler F.E., Q. Sun, J. Li, J.M. Tiedje. 2000. "16S rRNA Gene-Based Detection of Tetrachloroethene-Dechlorinating Desulfuromonas and Dehalococcoides Species." *Appl. Environ. Microbiol.* 66(4): 1369-1374.

Major, D.W., E.E. Cox, E.A. Edwards, and P. Hare. 1995. "Intrinsic Dechlorination of Trichloroethene to Ethene in a Bedrock Aquifer." In: R.E. Hinchee, J.T. Wilson, D.C. Downey (Eds.), *Intrinsic Remediation*, Battelle Press, Columbus, OH. p.197-203.

Major, D.W., M.L. McMaster, E.E. Cox, E.A. Edwards, S.M. Dworatzek, E.R. Hendrickson, M.G. Starr, J.A. Payne, and L.W. Buonamici. 2002. "Field Demonstration of Successful Bioaugmentation to Achieve Dechlorination of Tetrachloroethene to Ethene." *Environ. Sci. Technol.* 36(23):5106-5116.

Martin, R.E., E.J. Bouwer, and L.M. Hanna. 1992. "Application of Clean-Bed Filtration Theory to Bacterial Deposition in Porous Media." *Environ. Sci. Technol.* 26(5):1053-1058.

Maymo-Gatell, X., Y.-T. Chien, J.M. Gossett, and S.H. Zinder. 1997. "Isolation of a Bacterium that Reductively Dechlorinates Tetrachloroethene to Ethene." *Science*. 276:1568-1571.

Petrich, C.R., K.E. Stormo, D.B. Knaebel, D.R. Ralston, and R.L.O. Crawford. 1995. "A Preliminary Assessment of Field Transport Experiments Using Encapsulated Cells." In R.E. Hinchee, J. Fredrickson, and B.C. Alleman (Eds), *Bioaugmentation for Site Remediation*. Battelle Press, Columbus, OH. p. 237-244.

Rogers, B., and B.E. Logan. 2000. "Bacterial Transport in NAPL-contaminated Porous Media." *Jour. Env. Eng.* 126(7)657-666.

Ryan, J.N., and M. Elimelech. 1996. "Review: Colloid Mobilization and Transport in Groundwater." *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 107:1-56.

Salanitro, J.P., P.C. Johnson, G.E. Spinnler, P.M. Maner, H.L. Wisniewski, and C. Bruce. 2000. "Field-Scale Demonstration of Enhanced MTBE Bioremediation through Aquifer Bioaugmentation and Oxygenation." *Environ. Sci. Technol.* 34(19):4152-4162.

Seagren, E.A., B.E. Rittman, and A.J. Valocchi. 1994. "Quantitative Evaluation of the Enhancement of NAPL-Pool Dissolution by Flushing and Biodegradation." *Environ. Sci. Technol.* 28:833-839.

Semprini, L., P.K. Kitanidis, D.H. Kampbell, and J.T. Wilson. 1995. "Anaerobic Transformation of Chlorinated Aliphatic Hydrocarbons in Sand Aquifer Baswed on Spatial Chemical Distributions." *Wat. Res. Res.* 31(4):1051-1062.

Shellenberger, K., and B.E. Logan. 2002. "Effect of Molecular Scale Roughness of Glass Beads on Colloidal and Bacterial Deposition." *Environ. Sci. Technol.* 36(2):184-189.

Sholz-Muramatsu, H., A. Neumann, H. Messmer, E. Moore, and G. Diekert. 1995. "Isolation and characterization of *Dehalospirillum multivorans* ge. Nov. sp. nov., a Tetrachloroethene-utilizing, Strictly Anaerobic Bacterium." *Arch. Microbiol.* 163:48-56.

Stephan, R.J., K.L. Sperry, M.T. Walsh, S. Vainberg, and C.W. Condee. 1999. "Field-Scale Evaluation of In Situ Bioaugmentation of Chlorinated Solvents in Groundwater." *Environ. Sci. Technol.* **33**(16):2771-2781.

Streger, S.H., S. Vainberg, H. Dong, and P.B. Hatzinger. 2002. "Enhancing Transport of Hydrogenophaga flava ENV735 for Bioaugmentation of Aquifers Contaminated with Methyl tert-Butyl Ether." *Appl. Environ. Microbiol.* 68(11):5571-5579.

Sun, B., B.M. Griffin, H.L. Ayala-del-Rio, S.A. Hashshman, and J.M. Tiedje. 2002. "Microbial Dehalorespiration with 1,1,1-Trichloroethane." *Science.* 298:1023-1025.

Suthersan, S.S. 2001. *Natural and Enhanced Remediation Systems*. Boca Raton: Lewis Publishers.

Tani, K., M. Muneta, K. Nakamura, K. Shibuya, and M. Nasu. 2002. "Monitoring of *Ralstonia eutropha* KT1 in Groundwater in an Experimental Bioaugmentation Field by In Situ PCR." *Appl. Environ. Microbiol.* 68(1): 412-416.

Taylor, R.T., and M.L. Hanna. 1995. "Laboratory Treatability Studies for Resting-Cell In Situ Microbial Filter Bioremediation." In R.E. Hinchee, J. Fredrickson, and B.C. Alleman (Eds), *Bioaugmentation for Site Remediation.* Battelle Press, Columbus, OH. p. 15-29.

Wilson, R.D., D.M. Mackay, and K.M. Scow. 2002. "In Situ MTBE Biodegradation Supported by Diffusive Oxygen Release." *Environ. Sci. Technol.* 36(2): 190-199.

Witt, M.E., M.J. Dybas, R.M. Worden, and C.S. Criddle. 1999. "Motility-Enhanced Bioremediation of Carbon Tetrachloride-Contaminated Aquifer Sediments." *Environ. Sci. Technol.* 33(17):2958-2964.

Yang, Y., and P.L. McCarty. 2000. "Biologically Enhanced Dissolution of Tetrachloroethene DNAPL". *Environ. Sci. Technol.* 34(14): 2979-2984.

Yang, Y., and P.L. McCarty. 2002. " Comparison Between Donor Substrates for Biologically Enhanced Tetrachloroethene DNAPL Dissolution." *Environ. Sci. Technol.* 36(15): 3400-3404.

Yao, K.-M., M. Habibian, and C.R. O'Melia. 1971. "Water and Waste water Filtration: Concepts and Applications." *Environ. Sci. Technol.* 5(11):1105-1112.

Appendix D: Microcosm Report

Draft Report for Microcosm Testing at NAS Fallon, NV

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Introduction

Chlorinated ethenes such as tetrachloroethene (PCE) and trichloroethene (TCE) are some of the most common groundwater contaminants found at United States Department of Defense (DoD) facilities. In addition these compounds are persistent under most natural geochemical conditions. Remediation of these contaminants through biodegradation of the chlorinated ethenes is a promising alternative at many of DoD sites.

Reductive dechlorination is the primary pathway for biodegradation of chlorinated solvents. Through this pathway, the chlorine atoms on the ethenes are sequentially replaced by hydrogen atoms through a biologically-mediated process. Generally, the hydrogen is generated through fermentation of an electron donor. Although many microorganisms are capable of mediating the reductive dechlorination process, only *Dehalococcoides ethenogenes* is known to completely reduce PCE and TCE to ethene. Unfortunately, at sites that lack the presence of this microorganism, the reductive dechlorination process stalls at *cis*-1,2-dichloroethene (DCE). Therefore, the application of enriched cultures containing *D. ethenogenes* or closely related microorganisms can be used to complete the reductive dechlorination process.

In 1999, a 31-week microcosm test was performed with soils from NAS Fallon to determine the possibility of successfully performing bioaugmentation to achieve complete reductive dechlorination of chlorinated ethenes at the site. The site at NAS Fallon was selected because a number of previous studies had been conducted at the site to demonstrate reductive dechlorination through enhanced biostimulation, and each of these studies resulted in incomplete dechlorination. The test site is adjacent to a former unlined fire training area that was contaminated primarily with fuels, but also low levels of chlorinated ethenes (maximum PCE concentrations of 100 μg/L).

The Research and Development group at General Electric performed the microcosm studies in their laboratories, and selected the Pinellas culture for augmentation of the microcosm bottles. The Pinellas culture had previously been demonstrated to achieve complete reductive dechlorination of chlorinated solvents at NAS Pinellas and Dover AFB.

Site Selection and Site Conditions

The site at NAS Fallon was the location of several studies to investigate the possibility of achieving complete reductive dechlorination through enhanced biostimulation. Each of these studies resulted in the incomplete (stalling at c-DCE) Dechlorination of the TCE and PCE. NAS Fallon was selected as the site for the bioaugmentation demonstrate to determine if the reductrive dechlorination process could be pushed to completion with the addition of the microbial culture.

The test site is adjacent to a former unlined fire training area that was contaminated primarily with fuels, but also low levels of chlorinated ethenes (maximum PCE concentrations of 100 μg/L). The training area was in use from the 1950s to 1988. The site contains five separate test lanes that remain from previous bioremediation projects. These lanes are oriented parallel to the groundwater flow direction and are hydraulically isolated to allow for comparison of five different testing scenarios.

Geologic conditions in the vicinity of the test site are as follows. Sandy sediment covers the site and extends to a depth of approximately 4 ft. Beneath the sandy surface cover is a layer of clay-rich silts and sands. This fine-grained layer is approximately 2 ft thick and appears to be laterally continuous across the site, gradually grading into a 12-ft-thick layer of medium-sized sands. The fine-grained layer and sand layer form an unconfined aquifer that is laterally continuous across the site. At the bottom of the unconfined aquifer is a sandy silt and clay layer of low permeability that has a thickness in excess of 5 ft and is nearly 20 ft thick across most of Site 1. This silt and clay layer provides a barrier between the unconfined surface aquifer and the deeper confined aquifer. The deeper aquifer is artesian to the ground surface.

Near the test area, the upper aquifer extends from approximately 6 to 18 ft bgs, and is primarily composed of medium to fine sand and silt-sized particles. Groundwater in the upper aquifer flows in a southeasterly direction with a hydraulic gradient of 4.2×10^{-4} ft/ft. Results of a pumping test and the hydraulic gradient were combined to calculate a groundwater velocity of 0.07 ft/day.

Although chlorinated ethene concentrations are present at sufficient levels to conduct the microcosm study, the site presents several obstacles for reductive dechlorination through bioaugmentation. The groundwater contains high total dissolved solids, very high sulfate concentrations (7,200 mg/L), and high levels of metals that may be toxic to microorganisms (e.g., arsenic, molybdate, borate).

Microcosm Setup and Testing Conditions

Microcosm experiments were prepared in 100-mL bottles using soil and groundwater from the site that had been initially sparged with nitrogen to remove the chlorinated and maintain anaerobic conditions. The bottles were then amended with PCE to achieve an initial concentration of 40 μM. The microcosm experiments were designed so that two electron donors (lactate and methanol), supplemental nutrients, and the Pinellas culture were added to the microcosms alone and in combination. Following the decrease in the PCE concentration to levels below 5 μM, the bottles were respiked with PCE about 195 days after initiating the tests.

The experimental matrix included two sets of microcosms where electron donors alone were added, two sets of microcosms where electron donors and nutrients were added together, and two sets of microcosms where electron donors, nutrients, and an active Pinellas culture were added, along with unamended, killed, and positive controls. Electron donors were added weekly at concentrations of 5.0 mM sodium lactate and 10.0 mM methanol. Nutrient-amended microcosms received a revised formula of anaerobic mineral medium consisting of potassium and ammonium salts, trace metals, and a vitamin mix. This medium was added only at the beginning of the test.

The methanol-fed microcosms (both the unaugmented and augmented microcosms) quickly demonstrated slow dechlorination of PCE to TCE without significant reduction of sulfate (methanol is not used extensively by sulfate-reducing bacteria). Throughout the 22-day experiment, dechlorination beyond TCE was insignificant.

The microcosm experiments were conducted for a total of 31 weeks, or 220 days. Throughout the study, the concentrations of the chloroethenes were monitored on a weekly basis. In addition, the concentrations of sulfate, sulfide, pH, cations, and anions were measured on a routine basis to evaluate their changes, and effects on the dechlorination process.

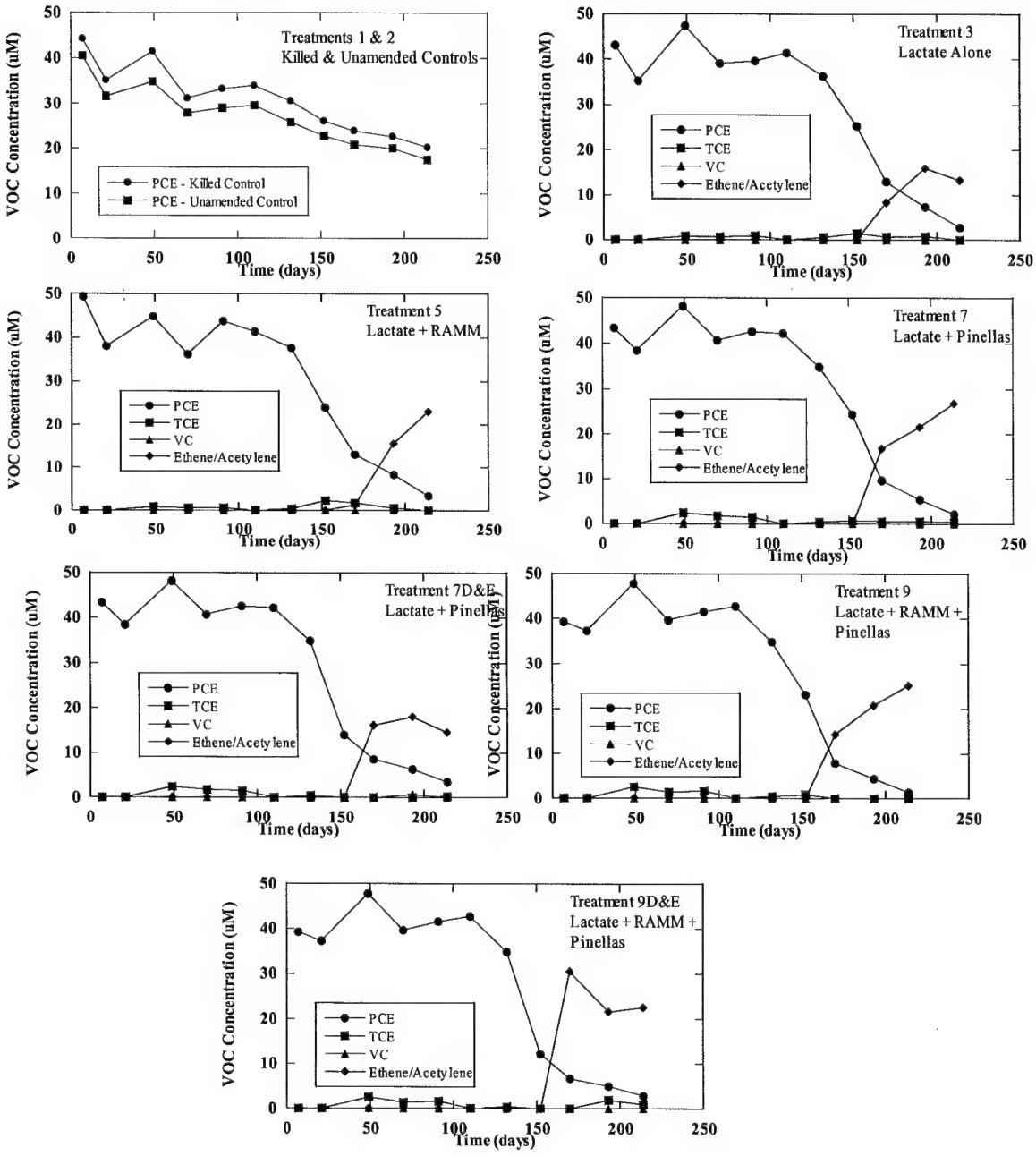
During week 19, ferrous chloride was added to the lactate-fed microcosms to precipitate the sulfide, and the sulfide concentrations were reduced to <5 mg/L in the bottles.

Results

Figures 1 presents the chloroethene concentrations throughout the study for the lactate-fed bottles. PCE concentrations in the lactate-fed microcosms (unaugmented and augmented) remained near the starting concentrations for approximately 19 weeks with slight transformation to TCE beginning near week 3. Betwwen the start of the test and Week 19, the majority of PCE loss was the result of volatilzation out of the microcosm bottles (as demonstrated in the killed and unamended controls).

The majority of the lactate in both the bioaugmented and non-bioaugmented bottles was used to reduce sulfate. Sulfate was reduced at a relatively constant rate of approximately 450 mg/L/week for the first 13 weeks. At week 16, sulfate had been depleted in several of the lactate-fed bottles, yet no significant dechlorination was occurring. It was then determined that the high sulfide concentrations (1,000 mg/L) in the microcosm bottles may have been inhibiting the reductive dechlorinating microorganisms. Presumably, the sulfide was present as free sulfide, due to the relatively high pH of the solution. During week 19, ferrous chloride was added to the lactate-fed microcosms to precipitate the sulfide, and the dissolved sulfide concentrations were reduced to <5 mg/L in the bottles.

Immediately following the ferrous chloride addition, the PCE concentrations declined at significant rates and both acetylene and ethene were produced as end products. After 12 weeks of the ferrous chloride addition, nearly all of the PCE had been removed from the microcosms and acetylene/ethene concentrations increased to 20 to 30 μM in all of the augmented and non-augmented bottles. The production of acetylene was believed to be the product of a non-biological reaction catalyzed by the iron sulfide produced during the ferrous chloride addition. Additionally, insignificant difference in the PCE reduction rate between the bioaugmented and non-augmented bottles suggests that the Pinellas culture would not greatly affect the reductive dechlorination rate or extent at the NAS Fallon site.



Figures 1-7. Choloroethene Concentrations in the Lactate-Fed Microcosms

Figure 2 presents the chloroethene concentrations from the methanol-fed microcosms. Methanol-fed microcosms were operated because it is generally understood the sulfate-reducing bacteria do not utilize methanol. These data indicate that reductive dechlorination of the PCE occurred throughout the study. However, the dechlorination process was limited to TCE. No degradation products past TCE were detected. Generally, the decrease in both the PCE and TCE concentrations over time was attributed to volatilization out of the microcosm bottles (as demonstrated in the kiled and unamended controls).

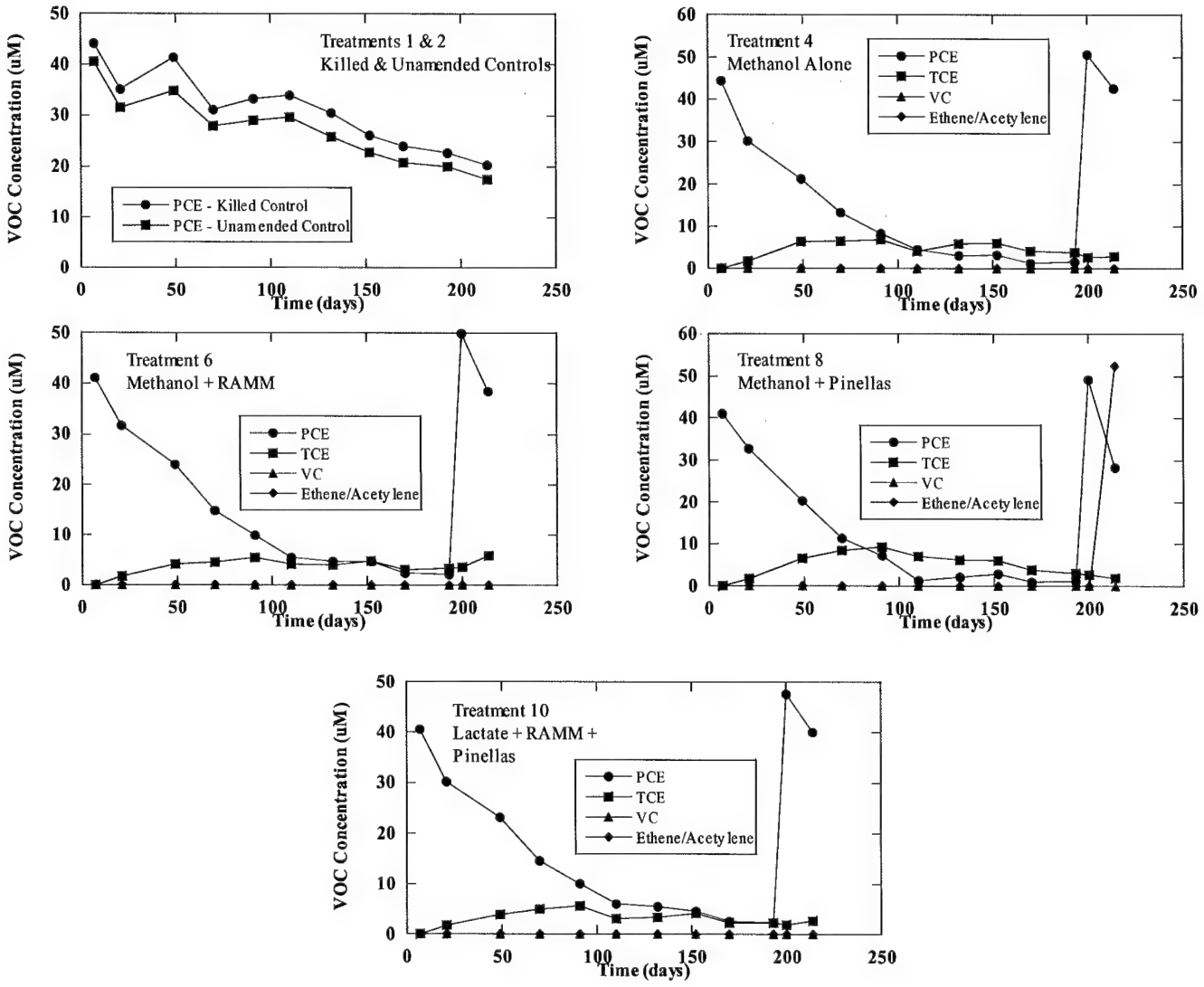


Figure 2. Chloroethene Concentrations in the Methanol-Fed Microcosms

In addition, the relatively small difference in the reduction of PCE between the controlled conditions and the test conditions indicate that the Pinellas culture had little effect on the reductive dechlorination process.

Conclusions

Due to the incomplete reductive dechlorination process in both the lactate-fed and methanol-fed microcosms and the relative small amount of transformation of PCE to TCE in both sets of microcosms, field-scale implementation of the bioaugmentation technology was not recommended. However, data from the lactate-fed microcosms indicate that complete reductive dechlorination of PCE to acetylene may be achieved through an iron sulfide-catalyzed reaction. With the high sulfate concentrations (>7,000 mg/L), it was suggested that the reduction of sulfate and the addition of ferrous chloride to the site could be performed to achieve complete reductive dechlorination at the site.

Cost and Performance Report
Demonstration of Bioaugmentation at Kelly AFB, Texas

Project funded by:

Environmental Security Technology Certification Program (ESTCP)
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June 10, 2004

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ABBREVIATIONS AND ACRONYMS

AFB	Air Force Base
bgs	below ground surface
c-DCE	*cis*-1,2-dichloroethene
DO	dissolved oxygen
DoD	Department of Defense
ESTCP	Environmental Security Technology Certification Program
GC/FID-ECD	Gas Chromatograph/Flame ionization Detector-Electron Capture Detector
gpm	gallons per minute
MCL	maximum contaminant level
MTBE	methyl-*tert*-butyl ether
O&M	Operation and Maintenance
ORP	oxidation-reduction potential
PCE	tetrachloroethene
ppb	parts per billion
PPE	personal protective equipment
PVC	polyvinyl chloride
RNA	remediation by natural attenuation
RTDF	Remediation Technology Demonstration Facility
TCE	trichloroethene
U.S. EPA	United States Environmental Protection Agency
VC	vinyl chloride
VFA	volatile fatty acid
VOC	volatile organic compound

1. Executive Summary

Chlorinated ethenes such as tetrachloroethene (PCE) and trichloroethene (TCE) are some of the most common groundwater contaminants found at Department of Defense (DoD) facilities. In addition to their common presence, these compounds are persistent under most natural geochemical conditions at these contaminated sites. Remediation of these sites through biodegradation of the chlorinated ethenes is a promising alternative at many of the sites. Reductive dechlorination is the primary pathway for biodegradation of chlorinated solvents. With this pathway, the chlorine atoms on the ethenes are sequentially replaced by hydrogen atoms through a biologically-mediated process. Generally, the hydrogen is generated through fermentation of an electron donor. Although many microorganisms are capable of mediating the reductive dechlorination process, only *Dehalococcoides ethenogenes* is known to completely reduce PCE and TCE to ethene. Unfortunately, *D. ethenogenes* is not present at all choroethene-contaminated sites and the reductive dechlorination process stalls at cis-1,2-dichloroethene (c-DCE). Under conditions such as these, the application of enriched cultures containing *D. ethenogenes* or closely related microorganisms is used to complete the reductive dechlorination process.

The primary objective of the demonstration was to determine if complete reductive dechlorination could be stimulated through the introduction of a culture known to contain halorespiring bacteria. Secondary objectives involved testing the robustness of the applied culture by depriving it of electron donor and adding sulfate to the system. Samples were collected at a frequency and analyses were performed to evaluate the objectives of the demonstration. The results of the chemical analyses indicated that the complete dechlorination was achieved through the addition of the microbial culture. Each of the performance objectives were met during the demonstration at Kelly Air Force Base (AFB). The data indicate that the KB-1 culture was capable of stimulating complete reductive dechlorination. In addition it was determined that the KB-1 culture was fairly robust with the elimination of the electron donor and the addition of the sulfate from/to the system.

In 1976, the United States Environmental Protection Agency (U.S. EPA) designated PCE and TCE as priority pollutants. The Safe Drinking Water Act Amendments of 1986 strictly regulate both of these compounds; each has a maximum contaminant level (MCL) in drinking water of 5 parts per billion (ppb) (U.S. EPA, 1996). When concentrations of these compounds at a contaminated site are too high, remedial action is required to lower the concentration and reduce the risk to human health and the environment.

Bioaugmentation was successfully demonstrated for achieving complete dechlorination at Kelly AFB where delivery of donor/nutrient amendments resulted in limited success. At Kelly AFB, dechlorination of PCE was demonstrated to hold up at *c*-DCE with only the addition of an electron donor. After the aquifer was augmented with KB-1, a prepared culture of halorespiring bacteria, complete dechlorination of PCE to ethene was observed.

Following the successful demonstration of the bioaugmentation technology, the robustness of the KB-1 culture was tested through the deprivation of electron donor and then the addition of sulfate. The objectives were to investigate the survivability of the KB-1 culture, evaluate any residual dechlorinating activity, attempt to reestablish the level of activity to pre-shutdown levels, and to stress the culture by adding sulfate. After approximately one year without the addition of the electron donor, gene probe analysis on groundwater samples collected across the augmented test plot all tested positive for the presence KB-1, and none of the samples from the non-augmented control plot tested positive. Complete PCE dechlorination was observed in one well inside the test plot suggesting that the KB-1 culture was utilizing a source of electron donor already in the groundwater. After the addition of the electron donor, complete reductive dechlorination was quickly observed in all of the wells.

Sulfate was added to establish an initial in-situ concentration of 600 mg/L. A significant amount of the sulfate was reduced, decreasing the concentration to 50 to 60 mg/L within 6 weeks. No apparent impact on the dechlorination activity was observed from the added sulfate.

The implications from these data are that (1) the KB-1 culture was very robust being able to compete with, and survive among, the indigenous microbial population, and (2) bioaugmentation may not require continuous attention following inoculation at sites where the natural attenuation requirements are met.

2. Technology Description

2.1 Technology Development and Application

Chlorinated solvents are widely used as solvents, cleaners, and degreasing agents. As a result of spills and past disposal practices, these compounds are contaminants in groundwater, soil, and sediments. Standard remedial approaches have proven to be ineffectual and costly at removing these substances from the environment. Within the last 15 years, basic research on natural microbial dechlorination mechanisms has suggested that the destruction of chlorinated compounds may be practically achieved at some sites by stimulating bacterial reductive dechlorination in the field.

Stimulation of microbial reductive dechlorination is achieved through the injection of electron-donating substrates and nutrients into the groundwater to produce proper reducing conditions. While stimulated biodegradation of chloroethenes may be an effective method of site remediation at many sites, there are instances where complete degradation of PCE and TCE to ethene is not possible through the addition of electron donors alone. In these cases, the degradation of PCE and TCE stops at c-DCE or vinyl chloride, resulting in the accumulation of these degradation components. The partial dechlorination of PCE and TCE may be caused by the absence of dechlorinating microorganisms (i.e., dehalorespiring microorganisms).

Cultures that contain phylogenetically-related organisms to *D. ethenogenes* have been produced for the application in the field. Examples of such cultures include the Pinellas culture and the KB-1 culture. A field demonstration of the Pinellas Culture was conducted at Dover AFB, and indicated that the dechlorination of c-DCE to ethene occurred only after the addition of the culture.

2.2 Process Description

For the technology demonstration, the bioaugmentation system was constructed as a plot that was hydraulically isolated. Hydraulic isolation of the plot was accomplished by recirculating water between one injection well and three extraction wells. To complete the installation of the test plots, one extraction well, three injection wells, and five monitoring wells were installed in an area of 20 ft by 30 ft.

The extracted groundwater was pushed into an equipment shed by the submersible pumps in the extraction wells, where the electron donors (methanol and acetate) were added to the groundwater stream to achieve a total concentration of 7.2 mM. The groundwater was then pumped back into the injection well. Groundwater recirculation rates were maintained near 3 gallons per minute (gpm) throughout the tests giving a residence time in the test cell of approximately 8 days.

In general, groundwater samples were collected every month during operation or when system operating parameters were modified. During each sampling event, groundwater was collected for volatile organic compound (VOC), volatile fatty acid (VFA), sulfate, nitrite, nitrate, bromide

(tracer), and dissolved gas analyses. In addition, samples were collected for gene probe analysis for detection of the KB-1 culture. During the sampling, the groundwater was monitored for several parameters in the field (i.e., pH, temperature, conductivity, dissolved oxygen (DO), oxidation-reduction potential, salinity, and turbidity). Groundwater sampling typically required 3 full days of labor for two technicians, but general operation and maintenance required daily monitoring of the system and collection of routine data.

The primary objective of the demonstration was to determine if complete reductive dechlorination could be stimulated through the introduction of a culture known to contain halorespiring bacteria. Secondary objectives involved testing the robustness of the applied culture by depriving it of electron donor and adding sulfate to the system. Samples were collected and analyses were performed at a frequency to evaluate the objectives of the demonstration. The results of the chemical analyses indicated that the complete dechlorination was achieved through the addition of the microbial culture.

Once the system has been installed, the labor requirements were relatively low. Daily monitoring of system operating conditions was required to ensure safe and consistent operation. With the system at Kelly AFB, fouling of the injection wells required regular surging and redevelopment of the wells. In addition, fouled recirculation tubing required replacement about every 3 or 4 months. In general, groundwater sampling was performed about every month or two during operation. Operation and monitoring of the system and sampling of the groundwater all could be performed in Level C personal protective equipment (PPE).

2.3 Previous Testing of the Technology

Demonstration of the bioaugmentation technology for the in situ treatment of chlorinated ethenes has been conducted at several sites from bench-scale to field-scale application. Results of these demonstrations and tests range from failure to complete success. Often with the successful demonstrations, the results are not conclusive that the complete reductive dechlorination is directly result of the addition of the culture. A White Paper prepared for Environmental Security Technology Certification Program (ESTCP) presents the state of the technology along with case studies of the demonstrations that have been performed.

2.4 Advantages and Limitations of the Technology

The advantages of bioaugmentation over traditional technologies for chlorinated solvent remediation, such as biostimulation or pump-and-treat, are cost and duration of cleanup project. Bioaugmentation is more cost effective than pump-and-treat technologies due to the high capital and operational costs of pump-and-treat systems. The installation and operation of the treatment system are the factors driving the cost of the pump-and-treat systems. Also, the duration of the remediation project may be shortened when bioaugmentation is used in place of a standard biostimulation process. The application of a culture to the contaminated aquifer likely would increase the biodegradation rates relative to simple biostimulation. Further, simple biostimulation may not achieve the remedial goals of complete reductive dechlorination to ethene.

The main advantages of bioaugmentation for remediation of chlorinated solvents include the following:

1. Bioaugmentation results in contaminant destruction, not simply phase transfer;

2. The technology utilizes the aquifer volume as an in situ bioreactor;

3. In situ destruction of the contaminant may relieve regulatory requirements associated with pumping followed by aboveground treatment;

4. In situ treatment minimizes water disposal and preserves water balance.

The main limitations of the bioaugmentation technology include the following:

1. The culture must establish a niche in the aquifer and be able to compete with the indigenous microorganisms for essential nutrients;

2. The application is limited to sites of sufficient permeability to allow manipulation of groundwater flow;

3. The overall effectiveness depends on the ability to distribute the culture adequately in the subsurface.

Bioaugmentation is an innovative technology and the status of regulatory acceptance is unknown.

3. Demonstration Design

3.1 Performance Objectives

The primary objective of the demonstration was to determine if complete reductive dechlorination could be stimulated through the introduction of a culture known to contain halorespiring bacteria. Secondary objectives involved testing the robustness of the applied culture by depriving it of electron donor and adding sulfate to the system. Samples were collected and analyses were performed to evaluate the objectives of the demonstration. The results of the chemical analyses indicated that the complete dechlorination was achieved through the addition of the microbial culture. Each performance objective was met during the demonstration at Kelly AFB. The data indicate that the KB-1 culture was capable of stimulating complete reductive dechlorination. In addition, it was determined that the KB-1 culture was fairly robust with the elimination of the electron donor and the addition of the sulfate from/to the system.

3.2 Selection of Test Site(s)

NAS Fallon was initially selected because a number of studies had been performed at the Site 1 location for reductive dechlorination and biostimulation. In addition, a test system was previously installed that could be used to conduct the demonstration. All of the studies were unsuccessful at achieving dechlorination to ethene. In some of these studies, the reductive dechlorination process could not be initiated.

After complete dechlorination could not be achieved at NAS Fallon in the microcosm tests using the Pinellas culture, it was decided that testing should be conducted at Kelly AFB, where bioaugmentation had successfully been demonstrated. At Kelly AFB, the objective was to determine the robustness of the KB-1 culture that was used at the site. At Kelly AFB, depriving the culture of electron donor for over a year would test the robustness of the culture. If the culture successfully rebounded and dechlorination was started again, the dechlorination process would be perturbed with the addition of sulfate to the test plot.

3.3 Test Site/Facility History/Characteristics

The location for the demonstration is situated in the courtyard of Building 360. The demonstration site was selected for the original bioaugmentation study based on the presence and concentrations of the contaminants, access to an existing test infrastructure, hydrogeology/ geology of site, site logistics (site access, electrical power, water, etc.). The site was selected for this demonstration because the existing infrastructure and data gathered to date provided the basis for the bioaugmentation study, and allows for additional studies to further enhance the understanding of the underlying principles of the technology and how various operational/environmental considerations impact the technology's performance.

The geology in the vicinity of the test site consists of unconsolidated alluvial deposits that have been deposited on the top of the undulatory erosional surface of the Navarro Clay (see Figure 3-1). The alluvial deposits consist of gravel, sand, silt, and clay, ranging in thickness from 20 to

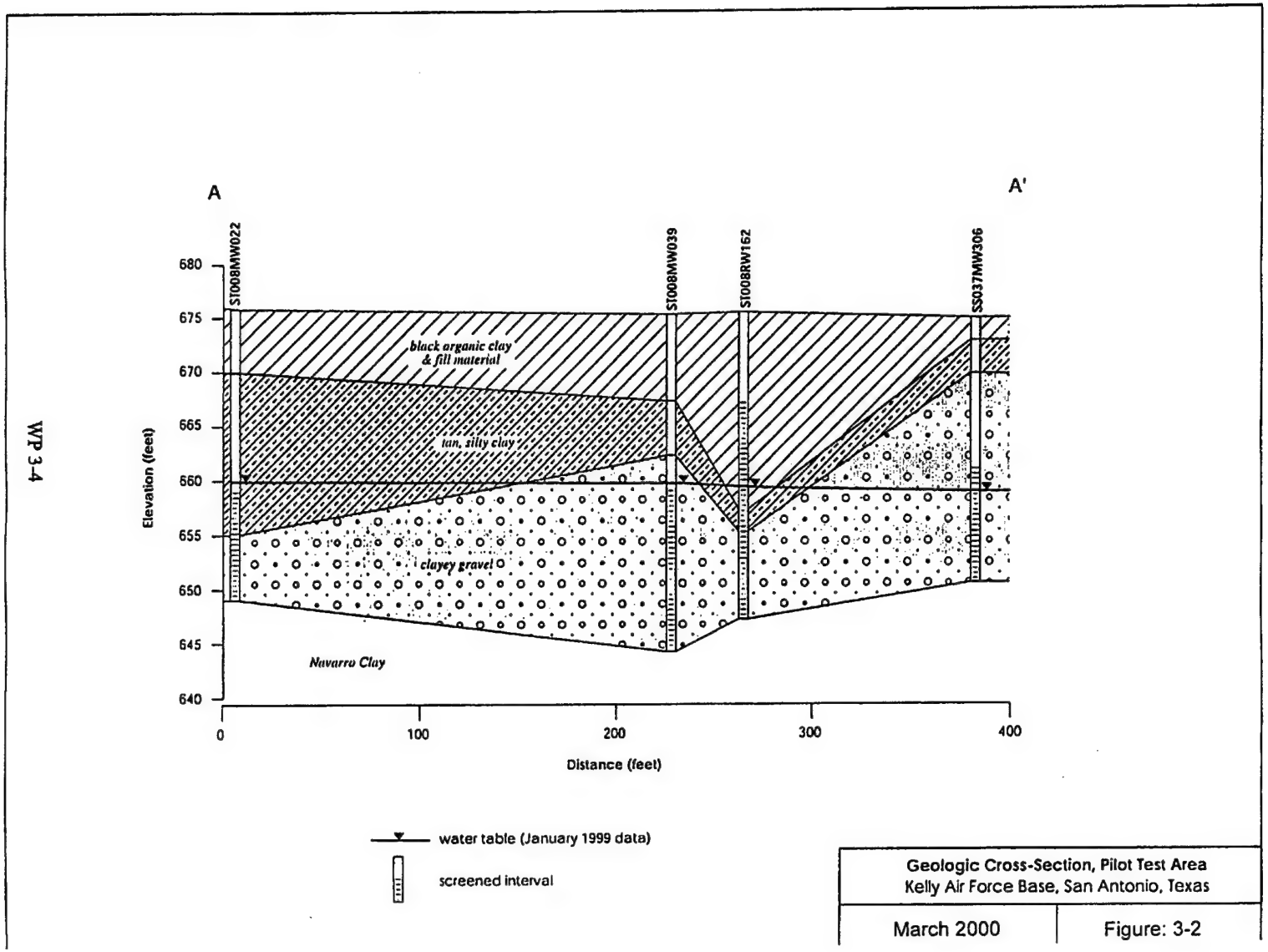

Figure 3-1. Cross-Sectional Diagram of the Surface Geology at Site 360, Kelly AFB

40ft. From the surface downward, the geology typically consists of 1 to 4 ft of black organic clay, 6 to 16 ft of tan silty, calcareous clay; and 4 to 20 ft of clayey limestone and chert gravel (denoted as clayey/gravel). The surface of the Navarro Clay is irregular and characterized by ridges and channel-like depressions.

Groundwater in the area of the demonstration site is primarily present in the limestone/chert layer. The water table is approximately 15 to 20 ft below ground surface (bgs), and the saturated thickness is between 5 to 12 ft. Generally, groundwater flow is to the southwest with a flow velocity of approximately 0.3 ft/day. The regional water table gradient is approximately 0.003.

VOCs in the site groundwater consist primarily of PCE, TCE, and their degradation products *c*-DCE and vinyl chloride (VC). Total chlorinated ethene concentrations in the groundwater exceed 8,000 µg/L.

3.4 Physical Set-up and Operation

Each plot has a total of nine wells: one injection well, three extraction wells, and five monitoring wells. Figures 3-2 and 3-3 contain cross-sectional and plan views of the test systems, respectively. Three of the monitoring wells (B-X wells) are aligned along the center of the plot parallel to the groundwater flow direction and located at a distance of 8, 12, and 22 ft downgradient of the injection well. The other two monitoring wells (T-X wells) are aligned perpendicular to groundwater flow, and were initially installed to be outside the zone of influence of the system. Each of the wells in both plots are completed to a depth of 25 ft bgs and were screened from 15 to 25 ft to reduce the opportunity for aeration and increased oxygen concentrations of the groundwater as it moved through the treatment system.

An injection/extraction process was used hydraulically isolate the test and control plots. The injection/extraction rates were the same as those used during the Remediation Technology Demonstration Facility (RTDF)/GeoSyntec project (approximately three gpm). These injection/ extraction rates were calculated by GeoSyntec using a groundwater modeling program and were demonstrated to have adequate isolation of the test cells and allow for a reasonable residence time in the cells during the RTDF/GeoSyntec project. Groundwater was extracted from the extraction wells using Grundfos submersible pumps and injected into the injection well after the addition of the amendments (electron donor, nutrients, etc.). The groundwater was pumped through a mobile shed where the nutrients were injected into the water stream using piston-style metering pumps.

Discussion of the operational conditions and periods of operation is presented in Section 4.1 (Performance Data) of this document.

3.5 Sampling/Monitoring Procedures

Groundwater samples were collected throughout all phases of the demonstration to evaluate the performance of the bioaugmentation technology at the Kelly AFB site. A peristaltic pump was used to purge 3 well volumes of water out of each well. The purged groundwater was passed through an inline flow through cell and then into a waste container. While the water was being

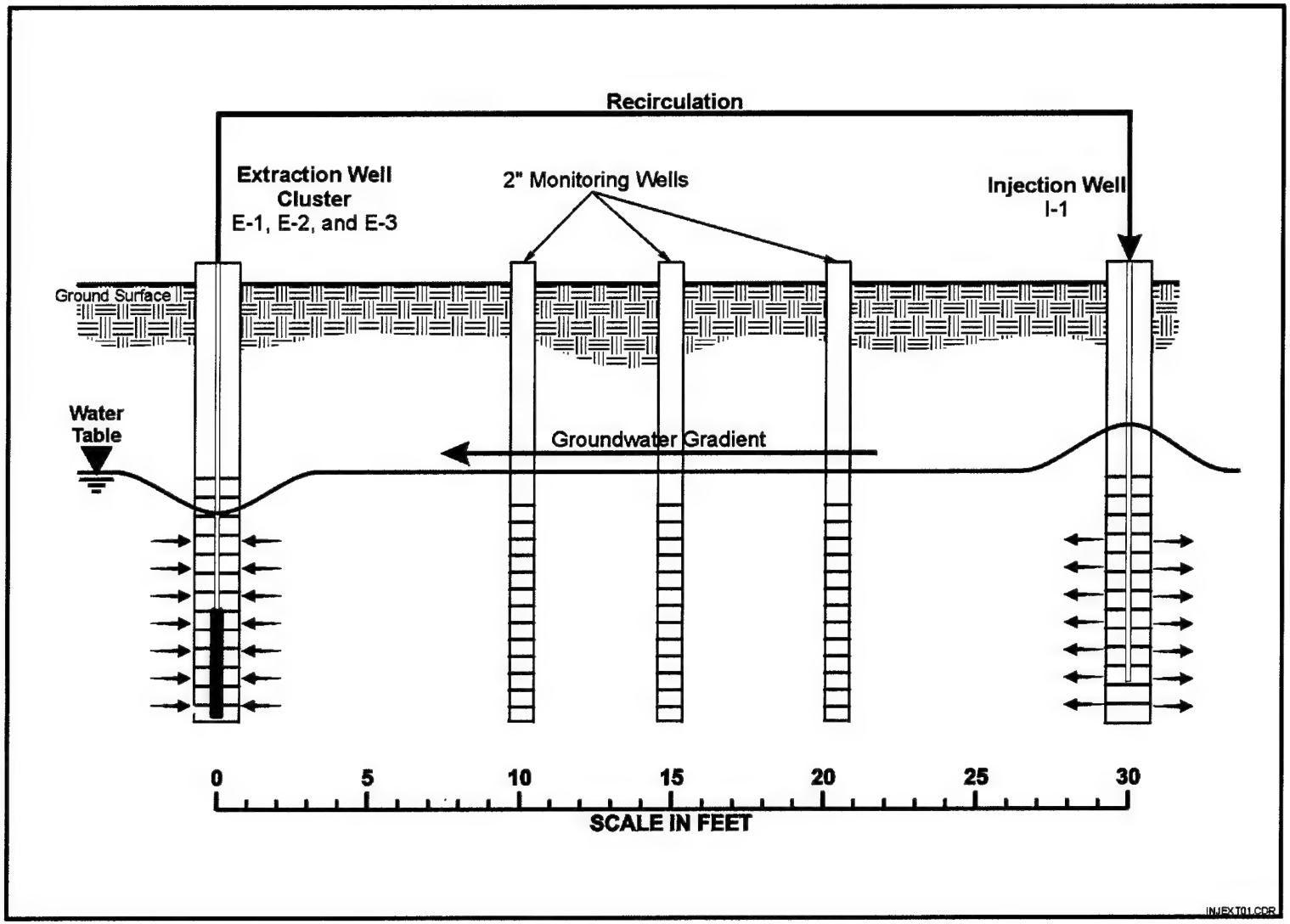

Figure 3-2. Cross Sectional Diagram of Test and Control Plots at Kelly AFB, TX

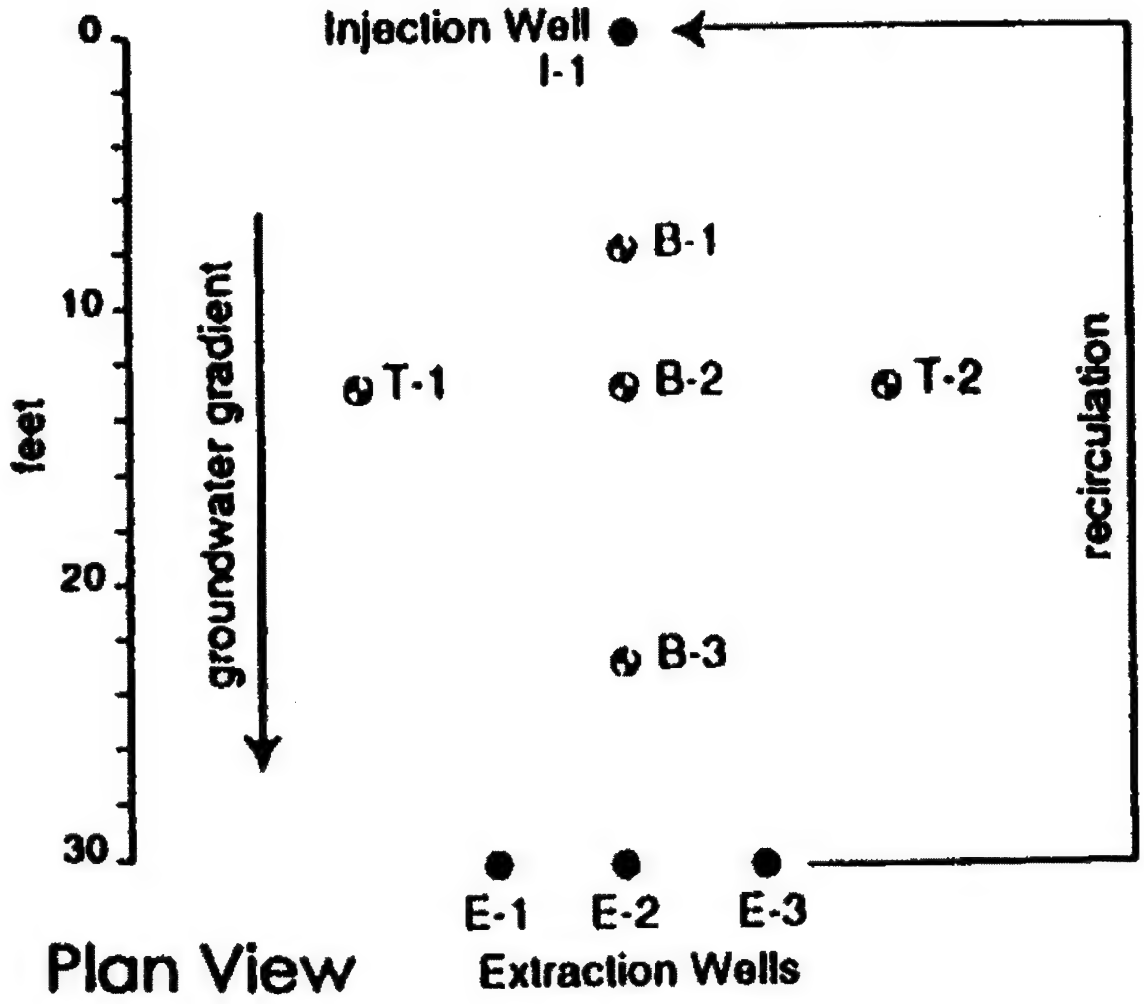

Figure 3-3. Plan View of the Test Plot at Site 360, Kelly AFB

purged, a Water Quality Meter was placed inside the flow-through cell and was used to measure the pH, conductivity, turbidity, DO, temperature, salinity, and oxidation-reduction potential (ORP) of the water. Once the purged water was removed from the well, the appropriate bottles were used to collect the samples of water. The VOC samples were preserved with HCl, and the samples were then packed with ice in a cooler and shipped to Alpha Analytical Laboratories for analyses. A complete list of analyses, standard methods, hold times, and location of analysis is presented in Table 3-1.

The samples were analyzed both in the field and in the laboratory, depending on the specific parameter being measured (Table 3-1). Groundwater samples were collected prior to starting the system to obtain baseline analyses. These samples were analyzed at a laboratory for PCE, TCE, DCE, VC, ethene, ethane, methane, VFAs, bromide, nitrate, nitrite, and sulfate. Following the startup of the system, groundwater samples were collected to measure the effects of the experimental parameters that were adjusted, and these samples also were analyzed for the laboratory and field parameters

In addition to the groundwater samples that were collected for chemical analyses, groundwater samples were collected to monitor the transport and survivability of the microbial culture through the test cells. These samples were collected prior to the start of the test to obtain baseline conditions. After the initiation of the demonstration, additional samples were be collected evaluate the migration and survivability of the microbial population during the test. The samples were sent to DuPont, for analysis using gene probe assaying to detect the culture.

Table 3-1. Analytical Methods

Measurement	Method	Instrumentation	Analysis Location
Critical Measurements			
PCE, TCE, *c*-DCE, VC,	SW 846 Method 8260B	Gas Chromatograph/ Flame Ionization Detector-Electron Capture Detector (GC/FID-ECD)	Laboratory
Ethene, Ethane, and Methane	EPA Standard Procedure (SOP)	GC FID	Laboratory
Volatile Fatty Acids (electron donor)	EPA (SOP)	GC/FID	Laboratory
Sulfate	EPA Method 300	Ion Chromatograph/ Conductivity Detector	
Bromide	EPA Method 300	Ion Chromatograph/ Conductivity Detector	Laboratory
Non-critical Measurements			
Nitrate, Nitrite, and Sulfate	EPA Method 300	Ion Chromatography/ Conductivity Detector	Laboratory
Bromide	Direct Reading	Bromide-Specific Electrode	Field
Dissolved Oxygen (DO)	Direct Reading	DO Probe	Field
pH	Direct Reading	pH Probe	Field
Conductivity	Direct Reading	Conductivity Meter	Field
Fe^{+2}	Hach Test Kit	Colorimeter	Field

4. Performance Assessment

4.1 Performance Data

Table 4-1 presents the chloroethene and ethene molar distributions in percent (of the compound per total molar chloroethene/ethene concentration) over the duration of the testing at Kelly AFB. These data are the average concentration of each ethene species from every well that was sampled. Samples were collected from before the system was started until the system was turned off (after the sulfate was added to the test plot). Dates that the system conditions were modified are as follows:

Baseline sampling and start of the system	November 12, 1999
Start electron donor addition	February 9, 2000
Addition of culture	May 6, 2000
Stop electron donor addition	September 25, 2000
Die-off samples collected	August 23, 2001
Start addition of sulfate (3.6 mM)	March 9, 2002
Start addition of sulfate (7.2 mM)	July 19, 2002

The changes in the chloroethene distribution relative to the modification in system operating conditions demonstrate the effect of the modification on the reductive dechlorination potential. The baseline distribution of the chloroethenes (11/12/99) indicated that PCE was the dominant chloroethene species and that limited reductive dechlorination was occurring through the presence of c-DCE. Following the addition of the electron donor, the chloroethene concentrations are affected by limited reductive dechlorination (i.e., the PCE concentrations decrease while the c-DCE concentrations increase). Complete dechlorination does not occur until after the test plot was bioaugmented on May 6, 2000. Within 72 days of the addition of the culture, ethene is detected in the test plot and the PCE, TCE, and c-DCE are near the lowest levels observed during the demonstration. These data indicate that the addition of the KB-1 culture promoted complete reductive dechlorination.

After demonstrating the effects of bioaugmentation for the potential to promote complete reductive dechlorination, the system was shut down and the addition of the electron donor was stopped on September 25, 2000. Groundwater samples were collected from the test plot on August 23, 2001 to determine the effects of eliminating the electron donor for one year on the populations of the KB-1 culture and the reductive dechlorination process. The microbial analyses and the distribution of chloroethenes indicated that the KB-1 culture was present and complete dechlorination was still occurring.

Sulfate was added to the system at 3.6 mM on March 9, 2002 to determine if the competitive use of the electron donor between the chloroethenes and sulfate would limit the reductive dechlorination occurring in the test plot. Data generated after May 9, 2002 indicate that the addition of sulfate did not significantly affect reductive dechlorination.

Table 4-1. Distribution of Chloroethene over Time

Date	Distribution of Chloroethene/Ethene (%)				
	PCE	TCE	c-DCE	VC	Ethene
11/12/99	72.5	1.6	25.7	0	0
2/15/00	73.0	1.3	25.6	0	0
3/16/00	68.6	2.6	28.7	0	0
5/3/00	16.3	1.4	82.3	0	0
5/22/00	21.5	11.4	66.5	0.5	0
6/5/00	18.4	19.1	62.4	0.1	0
6/27/00	12.7	2.7	83.0	1.6	0
7/17/00	10.2	0.7	76.3	8.4	4.4
8/7/00	10.0	0.6	32.5	15.9	41.0
8/29/00	10.7	0.5	20.7	8.9	59.2
9/25/00	9.0	0.4	10.2	4.0	76.5
8/23/01	21.3	1.8	45.8	17.5	13.5
10/11/01	8.6	0.8	70.9	19.8	0
11/7/01	19.8	0.8	14.4	9.6	55.4
11/28/01	15.3	0.8	18.3	9.5	56.1
12/18/01	16.9	0.9	19.0	8.9	54.3
3/19/02	7.9	1.2	40.9	50.0	NT
4/25/02	3.9	0.9	32.9	16.8	45.5

4.2 Performance Criteria and Performance Assessment

Table 4-2 presents the criteria that were used to assess the performance of the technology during the demonstration. The performance criteria are defined as primary or secondary depending on the importance to evaluating the performance of the technology.

The effectiveness of the bioaugmentation technology at achieving complete dechlorination was achieved by comparing the results produced in the test plot to those generated from the operation of a control plot within the same plume. The operating conditions and electron donor addition were same for both the control and test plots. In addition, prior to the addition of the culture, the system was allowed to operate until steady-state conditions had been achieved.

As was done with the testing of the overall bioaugmentation technology, the effects of eliminating the electron donor and the addition of sulfate were examined with the comparison of the results in the test plot with those in the control plot. Steady-state conditions also were achieved prior to modifying the conditions (i.e., electron donor and sulfate addition) in the test plot.

Table 4-2. Performance Criteria for the Bioaugmentation Demonstration

Performance Criteria	Description	Primary or Secondary
Contaminant Reduction	This technology is designed to reduce chloroethene contamination through sequential dechlorination to produce ethene as a final product.	Primary
Contaminant Mobility	Through the sequential dechlorination process, the mobility of the products is not substantially increased or decreased.	Secondary
Hazardous Materials	If successful conducted, no hazardous materials would remain or be introduced through the implementation of the bioaugmentation technology. However, the use of bioaugmentation may prevent the formation and accumulation of more hazardous compounds, such as vinyl chloride that may be produced during biostimulation.	Primary
Process Waste	The use of this technology does not produce any process waste.	Secondary
Factors Affecting Technology Performance	The bioaugmentation technology is affected by groundwater geochemistry, hydrogeologic characteristics of the site, and survivability of the culture. **Geochemistry:** Sulfate inhibits the reductive dechlorination process. High or low pH, high salinity or high levels of metals may adversely affect the introduced culture. **Hydrogeology:** Low permeability may limit distribution of culture. High levels of organic matter may limit distribution of the culture, but may provide a source of electron donating substrate. **Survivability of the Culture:** Competition of the culture with the indigenous microbial population may affect the survival rate of the applied culture. Moderately alkaline conditions may favor the survival of the culture. Factors affecting the performance of the technology are discussed in greater depth in the Current State of the Bioaugmentation Technology.	Primary
Reliability	The bioaugmentation technology as it was applied during the demonstration was relatively reliable. Problems were encountered with the recirculation pumps. However, this style of pumping would be eliminated during full-scale operation	Secondary
Ease of Use	Both at the demonstration-scale and with full-scale operation the technology is relatively easy to use. The only pieces of equipment that are used are pumps for the injection of electron donor.	Secondary

Table 4-3. Expected Performance and Performance Confirmation Methods (Continued)

Performance Criteria	Description	Primary or Secondary
Versatility	This technology is likely very versatile depending on the culture applied and the target contaminant. Cultures have been produced to treat chloroethenes, MTBE, petroleum hydrocarbons, and chlorinated methanes, and PCBs. The bioaugmentation technology has long been used in the wastewater treatment systems.	Primary
Maintenance	Moderate maintenance was required for the technology demonstration. Daily monitoring of the system equipment and the water levels in the injection/extraction were required to ensure the injection well would not overflow and the water levels in the in the extraction well was not lowered beneath the top of the screen. Also, pumps, and electron donor solutions needed to be monitored to ensure continuous flow.	Secondary
Scale-Up Constraints	The widespread application of the culture represents the greatest challenge with the scale-up of the technology. Direct contact between the culture and the contaminant is imperative for success of the technology. As the culture is injected in a well, the contaminants are pushed in front of the microbial culture. Therefore, the use of an in situ biobarrier may be the most effective method to provide intimate contact between the contaminants and the culture.	Primary

A total of 15 sampling events were conducted over the course of the bioaugmentation study at Kelly AFB. In general, the sampling events occurred just prior to and then shortly after making a modification to the system test conditions. Following the sampling events near the modification, samples were collected about every month to investigate long-term effects of the system changes. During each sampling event, a complete suite of analyses was performed to determine the effects of the system modifications. For example, specific analyses were performed (i.e., microbial gene probe) to confirm the presence of the KB-1 culture in areas where complete dechlorination was occurring.

Table 4-3. Expected Performance and Performance Confirmation Methods

Performance Criteria	Expected Performance Metric (pre demo)	Performance Confirmation Method	Actual (post demo)
PRIMARY CRITERIA (Performance Objectives) (Qualitative)			
Contaminant Mobility	No change	Not measured	Uncertain
Faster Remediation	Achieve complete dechlorination and	Monitor chloroethene concentrations in the	The bioaugmented plot achieved complete

Table 4-3. Expected Performance and Performance Confirmation Methods (Continued)

Performance Criteria	Expected Performance Metric (pre demo)	Performance Confirmation Method	Actual (post demo)
	reduce remediation time	test and control plots	dechlorination, while the control plot did not. Therefore, bioaugmentation would decrease remediation times relative to biostimulation and natural attenuation
Ease of Use	Minimal operator training required	Monitor labor requirements	Minimal operator training was required for continuous operation.
PRIMARY CRITERIA (Performance Objectives) (Quantitative)			
Feed Stream - Recirculation rate	2gpm	Continuous rotometer	Generally, achieved 2 gpm, but pump failure and water levels in the injection well reduced flowrates at times.
- Electron donor injection rate	3.6 mM (time-weighted)	Calibrated metering pumps	Achieved accurate injection levels
- Contaminant concentration	Total chloroethene 10 µM	U.S. EPA Method 8260	Maintained good mass balance
Target Contaminant - Percent reduction - Regulatory standard			
Hazardous Materials - Generated	None	Analysis for VC	Vinyl chloride was detected as a transient species
Process Waste - Generated	None	Observation	None detected
Factors Affecting Performance - Geochemistry	Geochemical conditions may limit survival of culture and dechlorination process	Analyze geochemical conditions (various methods), chloroethene concentrations (U.S. EPA Method 8260) and microbial populations	Natural water chemistry did not inhibit culture growth, nor did it prevent reductive dechlorination. Limited amounts of added

Table 4-3. Expected Performance and Performance Confirmation Methods (Continued)

Performance Criteria	Expected Performance Metric (pre demo)	Performance Confirmation Method	Actual (post demo)
			sulfate (3.6 mM) did not affect dechlorination.
- Hydrogeology	Hydrogeologic conditions may limit distribution of culture	Performed tracer tests with microbial analyses	Hydrogeology at the site did not limit distribution of culture. The culture had populate the entire test cells within 3 months of injection
- Survivability	Lack of electron donor may kill culture	Eliminated electron donor addition, and monitored VFAs and microbial populations	This elimination of the electron donor addition did not stop reductive dechlorination process nor did the KB-1 culture die off
SECONDARY PERFORMANCE CRITERIA (Qualitative)			
Reliability	Limited shutdowns	Record Keeping	Moderate to high number of shutdowns due to pump failures, high groundwater levels, and fouling wells and tubing.
Safety - Hazards - Protective clothing	Chloroethenes Level C personal protective equipment (PPE)	Experience from demonstration operation	Level C PPE provided adequate protection
Versatility - Intermittent operation	Yes	Experience from demonstration operation	Intermittent operation did not negatively affect system operation
- Other applications	Yes		Technology may be used for other chlorinated species and MTBE depending on the culture applied
Maintenance - Required	Regular changing of tubing, development of	Experience from demonstration operation	Regular replacement of the tubing was required

Table 4-3. Expected Performance and Performance Confirmation Methods (Continued)

Performance Criteria	Expected Performance Metric (pre demo)	Performance Confirmation Method	Actual (post demo)
	the injection well		and development of the injection well was performed, but fouling was still a problem.
- Eliminated	None		
Scale-Up Constraints - Distribution of Culture	Widespread distribution of culture would be required for large-scale application	Monitored migration of culture throughout demonstration	The culture was spread throughout the test plot relatively quickly due to the operation of the recirculation system. For large-scale application, the culture may need to be used in a biobarrier form to get intimate contact between the culture, electron donor, and contaminants

4.4 Technology Comparison

It is difficult to compare the performance of the bioaugmentation technology with other innovative alternative technologies, such as biostimulation. Bioaugmentation may used to produce complete dechlorination that otherwise would not be achieved through simple biostimulation. From this standpoint, bioaugmentation may be the only option for meeting cleanup goals, if bioremediation is selected for the remedial action. Also, bioaugmentation may reduce cleanup times compared to biostimulation by eliminating the lag period between the initiation of electron donor injection and the onset of complete dechlorination, which may last several months in successful biostimulation projects. However, the time savings from using bioaugmentation is very difficult to predict. At some sites it may be less than one month at others it may be more than a year. The cost savings from reducing the lag time to achieve complete dechlorination is reduced by the by the cost of the culture and application of the culture. Because the reduction in the lag time is impossible to determine, it is difficult to compare bioaugmentation with biostimulation.

5. Cost Assessment

The bioaugmentation demonstration was conducted in two stages: a microcosm test (performed at NAS Fallon), and a full-scale demonstration conducted at Kelly AFB. The microcosm testing at NAS Fallon indicated the bioaugmentation would not be feasible at that site. Previous testing had been conducted at Kelly AFB; therefore, no microcosm testing was required prior to full-scale work at that site. Because microcosm testing is recommended prior to performing a full-scale remediation project, this cost assessment includes costing for both the microcosm and full-scale stages of the demonstration.

5.1 Cost Reporting

Throughout the course of this demonstration, the cost data were tracked to provide accurate cost information on the scale-up of the technology once it had been demonstrated. Costs associated with labor, consumable equipment, capital equipment (rented and purchased), subcontracted labor (Operation and Maintenance [O&M] providers), and purchased services (drillers and analytical) were tracked, and provide a basis for comparing bioaugmentation to other traditional technologies. Costs were tracked for both the microcosm and field-scale testing. The system used at Kelly AFB generally was established prior to the ESTCP testing at the site; therefore, some of the costs had to be estimated for the field scale testing of the technology.

The majority of the costs for bioaugmentation used to evaluate the cost performance of the system and to compare the costs of performing bioaugmentation to other technologies were obtained from the demonstration-scale tests. However, the majority of the system used during the demonstration had been constructed prior to the conduction of the demonstration, and these costs were estimated. For the full-scale implementation of the bioaugmentation technology, costs for the microbial culture were obtained from Regenesis, Inc. and the costs for microbial analysis were obtained from Sirem, Inc. Costs associated with performing pump-and-treat were estimated from previously performed projects. The majority of the costs for pump-and-treat were the installation of wells. Costs for performing the permeable reactive barrier technology were primarily obtained from the cost and performance report for this technology submitted to ESTCP.

5.1.1 Microcosm Testing. The cost to perform the microcosm testing option performed at NAS Fallon was estimated at $78,000. Table 5-1 shows the cost breakdown. During the microcosm testing, two conditions were tested: an unaugmented control and augmented test bottles. Both of these conditions were conducted in triplicate and at least biweekly analyses were performed on the bottles. The soil samples were collected from an average depth of 20 ft bgs. Although GE provided the culture, an estimated cost of $500 was used for GE to produce the culture.

5.1.2 Field Testing. The cost to complete a field test of bioaugmentation at Kelly AFB is presented in Table 5-2. The total cost of performing a field test of the bioaugmentation

technology was estimated at $255,936. Again, some of the costs associated with installation had to be estimated because the system had been used previously for bioremediation testing.

The layout of Kelly AFB consists of one injection well, three extraction wells, and six monitoring wells covering an area of approximately 30 ft by 20 ft. The total volume of groundwater treated by the demonstration system was approximately 40,000 gallons. Monitoring wells used for the demonstration were constructed of 2-inch polyvinyl chloride (PVC), and the injection and extraction wells were 4-inch PVC. The field trailers were used to store equipment and provided a location for the electron donor, tracer, and sulfate to be added to the system.

Mobilization costs included transporting the field trailers to the site and securing the trailers at the site. The majority of the site work costs include the construction costs for preparing the site, such as drilling and electrical installation. The labor and analytical costs are the dominant part of the variable costs, where the equipment and materials costs are much lower.

Table 5-1. Estimated Cost of Microcosm Testing

Activity	Unit Cost	Quantity	Cost
Microcosm Test Plan	$5 K	1	$5K
Microcosm Testing			
Soil Collection			
Labor	$2K	1	$2K
Travel	$3K	1	$3K
Drilling costs			
Mobilization	$1K	1	$1K
Drilling (20-ft deep)	$25/lf	100 lf	$2.5K
Waste disposal	$2K	1	$2K
Misc. (decontamination, etc.)	$1K	1	$1K
Consumables and supplies	$1K	1	$1K
	$0.5K	1	$0.5K
Conduct Testing			
Labor	15K	1	$15K
Analytical services			
VOCs	$100/sample	200	$20K
Data analysis	$5K	1	$5K
Reporting	$10K	1	$10K
Total Cost for Microcosm Testing			**$78K**

The estimated costs for performing the remediation effort at the scale of the demonstration is presented in Table 5-3. A cost of the remedial effort compared to the ESTCP demonstration indicates that the cost of the remedial effort would be approximately $72,000 less than performing a standard demonstration. The fixed costs (system installation costs) would be nearly the same for both the demonstration and the remedial effort. However, the variable costs for the remedial effort would likely be lower than the standard demonstration because of the limited

sampling and analysis. For the remedial project, samples could be collected on a quarterly basis. The labor costs decrease by about $25,000 for the remedial effort because the routine maintenance would still be required for the remedial effort. However, the duration of the remedial project likely would be less for the remedial project due to fact that the remedial goals would be achieved faster than the performance goals of the demonstration.

5.2 Cost Analysis

5.2.1 Cost Comparison. A typical technology for treating chlorinated solvent-contaminated sites is pump-and- treat. Pump-and-treat is a traditional technology for remediating sites with chlorinated solvent contamination. For full-scale bioaugmentation operation, the use of a biobarrier would likely provide the most effective method of aquifer remediation. A comparison of the use of a biobarrier and pump-and-treat over time is provided in Table 5-4.

Table 5-2. Costs for Field Demonstration at Kelly AFB, TX

Cost Category	Subcategory	Costs ($)
	FIXED COSTS	
1. CAPITAL COSTS	Mobilization/demobilization - Mobilization of trailers	 $1,000
	Demonstration Plan	$15,000
	Site work	$20,000
	Equipment Cost - Extraction/Metering Pumps - Manifold/Tubing	 $3,750 $600
	Installation - Drilling - Electrical	 $22,367 $5,000
		Subtotal $67,727
	VARIABLE COSTS	
2. OPERATION AND MAINTENANCE	Labor - Subcontractor - Battelle personnel	 $75,678 $20,312
	Materials and Consumables - Chemicals - Material	 $3,000 $5,000
	Travel costs	$9,250
	Culture Chemical/Biological Analyses	$10,000 $43,853
	Performance Data Analysis/Reporting	$11,454
	Trailer Rental	$9,600
		Subtotal $188,209
	TOTAL COSTS	
		TOTAL TECHNOLOGY COST : $255,936

Note: Base provided electrical utility.

Table 5-3. Costs for Field-Scale Demonstration

Cost Category	Subcategory	Costs ($)
	FIXED COSTS	
1. CAPITAL COSTS	Mobilization/demobilization - Mobilization of trailers	 $1,000
	Work Plan	$7,500
	Site work	$20,000
	Equipment Cost - Extraction/Metering Pumps - Manifold/Tubing	 $3,750 $600
	Installation - Drilling - Electrical	 $22,367 $5,000
		Subtotal $60,217
	VARIABLE COSTS	
2. OPERATION AND MAINTENANCE	Labor - Subcontractor - Battelle personnel	 $50,000 $5,000
	Materials and Consumables - Chemicals - Material	 $3,000 $5,000
	Travel costs	$5,000
	Culture Chemical/Biological Analyses	$10,000 $14,420
	Performance Data Analysis/Reporting	$11,454
	Trailer Rental	$9,600
		Subtotal $113,474
	TOTAL COSTS	
		TOTAL TECHNOLOGY COST : $173,691

Note: Base provided electrical utility.

Table 5-4. Costs Comparison for Field Demonstration at a Generic Site

Cost Category	Subcategory	Bioaugmentation Costs ($)	Pump-and-Treat Costs ($)
	FIXED COSTS		
1. CAPITAL COSTS	Mobilization/demobilization		
	- Mobilization of trailers	$1,000	$1,000
	Demonstration Plan	$25,000	$20,000
	Site work	$20,000	$100,000
	Equipment Cost		
	- Extraction/Metering Pumps	$6,000 $1,000	$4,000 $5,000
	- Manifold/Tubing	$0	$105,000
	- Treatment Equipment (Air Stripping/Catalytic Oxidizer)	$15,000	$0
	- Biological Culture		$15,000
	Installation		
	- Drilling with Disposal	$33,000	$83,000
	- Electrical	$10,000	$60,000
	Subtotal	$111,000	$378,000
	VARIABLE COSTS		
2. OPERATION AND MAINTENANCE	Labor (total)		
	- Subcontractor	$130,000	$390,000
	Materials and Consumables (total)		
	- Chemicals	$48,000	$0
	- Material	$24,000	$75,000
	- Electricity	$5,000	$25,000
	- Propane	$0	$20,000
	Chemical/Biological Analyses (total)	$63,000	$49,000
	Performance Data Analysis/Reporting	$11,000	$11,000
	Trailer Rental	$10,000	$10,000
	Subtotal	$291,000	$570,000
	TOTAL TECHNOLOGY COST :	$402,000	$948,000
			Quantity Treated: 814,000 gallons

The costs presented in the cost comparison were derived from the generic site with a 5 acre chlorinated ethene plume having dimensions of 300 ft by 700 ft. The depth to groundwater is set as 15 ft and the total depth of the aquifer is 25 ft.

For construction of a biobarrier, it was believed that 20 wells would be required across the leading edge of the plume. Each of these wells would be screened across the thickness of the saturated zone. The biological culture would be injected into each of the wells, and the desired

cell density (10^4 cells/ml) in the aquifer would be achieved through pumping and cell growth. It is estimated that approximately 25 L of the culture would need to be added to the system. The wells installed for the pump-and-treat system would be evenly spaced throughout the plume, and it was believed that 50 wells would be required to cover the plume.

The costs for equipment and materials are much higher for pump-and-treat primarily because of the costs of the air stripping and catalytic oxidizer systems. It was estimated that these components would be approximately $105,000. The only addition materials costs that bioaugmentation would have are with the biological culture (estimated at $15,000).

The variable costs for pump-and-treat are significantly higher than those for bioaugmentation. While it is expected that the duration of the pump-and-treat system would be half as long as the biobarrier system, a significant cost associated with pump-and-treat is the operation and maintenance. It was estimated that the treatment systems for the pump-and-treat system would require 60 hours per/week while the bioaugmentation system would require 10 hours per week. The analytical costs associated with the biobarrier are only slightly higher due to the microbial analyses.

The total costs of the technologies would be $402,000 for bioaugmentation and $948,000 for pump-and treat. The total volume of groundwater that would be treated would be approximately 814,000 gallons. Therefore the unit treatment costs for bioaugmentation and pump-and-treat would be approximately $ 0.50/gallon and $1.16/gallon, respectively.

The cost of implementing bioaugmentation through the use of a biobarrier were also compared to the implementation of a permeable reactive barrier with iron medium. The cost associated with the permeable barrier were obtained from the cost and performance report for Evaluating the Longevity and Hydraulic Performance of Permeable Reactive Barriers at DoD sites (ESTCP, 2003). Costs for the permeable had to be estimated because unit costs were not presented in the report. It was assumed that the reactive barrier used during this cost estimate would need to be approximately 3 times as large as the barrier used during the field demonstration at NAS Moffet Field. The total cost of the sheet pile was estimated from the NAS Moffet Field installation. The NAS Moffet Field system was approximately 7.5 times narrower than the fictitious site used for these cost estimates. Because both technologies rely on natural groundwater movement, the treatment times for both the bioaugmentation and reactive barrier technologies were the same.

Table 5-5. Cost Comparison of Bioaugmentation and Permeable Reactive Barrier

Cost Category	Subcategory	Bioaugmentation Costs ($)	Permeable Barrier Costs ($)
	FIXED COSTS		
1. CAPITAL COSTS	Mobilization/demobilization		
	- Mobilization of trailers	$1,000	$1,000
	Work Plan	$25,000	$25,000
	Site work	$40,000	$100,000
	Equipment Cost		
	- Extraction/Metering Pumps	$6,000	$0
	- Manifold/Tubing	$1,000	$0
	- Biological Culture	$15,000	$0
	Installation		
	- Drilling with Disposal	$33,000	$0
	- Electrical	$10,000	$0
	- Sheet Pile Installation	$0	$405,000
	- Reactive Barrier/iron medium	$0	$417,000
	Subtotal	$131,000	$948,000
	VARIABLE COSTS		
2. OPERATION AND MAINTENANCE	Labor		
	- Subcontractor	$130,000	$40,000
	Materials and Consumables		
	- Chemicals	$40,000	$0
	- Materials	$24,000	$0
	- Electricity	$5,000	$0
	Chemical/Biological Analyses	$55,000	$40,000
	Performance Data Analysis/Reporting	$11,000	$11,000
	Trailer Rental	$10,000	$10,000
	Subtotal	$275,000	$101,000
	TOTAL TECHNOLOGY COST :	$406,000	$1,049,000

Although the cost comparison in this report was made between bioaugmentation and pump-and treat and bioaugmentation and permeable reactive barriers, a comparison may be made between bioaugmentation and biostimulation. However, a comparison between bioaugmentation and biostimulation is more difficult because the cost difference is not easily defined. The benefit from applying a culture results from a potential decrease in remediation time, and the magnitude of this decrease is uncertain as well as site dependent. Therefore, the cost benefit from applying the bioaugmentation technology over biostimulation is uncertain.

5.2.2 Cost Drivers and Potential Cost Impacts. The costs provided for each testing option (i.e., microcosm or field test) were calculated under assumptions that were developed to describe a "typical" site. The actual costs for both microcosm testing and field testing would depend on site-specific requirements/ logistics. Due to the variability in site conditions, there is a large amount of uncertainty in the cost estimates used in this report. The variables that affect each approach and their potential impact are summarized in the following sections.

5.2.3 Cost Drivers. The single variable that could significantly impact the cost of conducting the microcosm tests is the depth of the contamination, which has a direct effect on the costs associated with collecting the aquifer core material, specifically the drilling, waste disposal, and labor costs. The costs presented in Section 5.1 assume a depth of 25 ft. Collection of cores from shallower sites would be somewhat less expensive, while collection of soil from deeper sites would obviously be greater. For example, if the contamination were located at 200 ft, the total cost of the microcosm test would increase on the order of $40,000. The drilling costs would increase by $22,000 and the disposal costs would increase by $20,000

The most significant cost variables for the field implementation of bioaugmentation are the hydraulic conditions at the site and the depth to the contamination. Lower hydraulic conductivities at a site would require a greater number of wells be used at the site to obtain relatively rapid distribution of the culture. Also, a greater number wells may be required to get even and rapid distribution of the electron donor and any nutrients. In general, a site with lower hydraulic conductivity would also require a longer period of operation, if the system relied on natural groundwater flow through the biobarrier, thus increasing operational costs. The impact that depth has on the costs, however, is much more pronounced than for the microcosm testing. Not only is the system installation cost impacted, but the cost of conducting the test is impacted as well. Implementing the bioaugmentation technology a 200-ft-deep site would result in a dramatic cost impact. The cost of labor for well installation would increase to $90,000, and the waste disposal would increase to $20,000. The materials costs would increase by four times due to install the system to the greater depth. The labor costs for conducting the test would increase primarily because of the need for increased sampling times.

5.2.4 Life Cycle Costs. For full-scale implementation of bioaugmentation, the capital costs and life-cycle costs are dependent on the design of the system used. As suggested previously, the most effective method of treating an aquifer with bioaugmentation likely would be a biobarrier. Capital costs for the installation of a biobarrier would be dependent on the depth of the aquifer and the lateral extent of contamination.

Operational costs would be relatively low due to the simplicity of the system. The bulk of operational costs would be associated with the regular sampling to ensure that the barrier is effectively treating the contaminated groundwater. Analysis would include chloroethene, dissolved gasses and VFA concentrations. The frequency of sampling and analysis would likely be dependent on the requirements of the overseeing regulatory agency.

Due to the relatively high capital cost for the installation of the biobarrier system, it would be recommended that microcosm or field treatability testing be performed prior to the full-scale implementation of the technology. If complete dechlorination to ethene is not observed in the microcosm or field-scale testing, full-scale operation of the technology should be reconsidered. Performing on small-scale testing should significantly reduce the liability associated with the partial dechlorination of PCE/TCE to another regulated compound, such as vinyl chloride.

Table 5-5 presents the life cycle costs for implementing the bioaugmentation technology in the biobarrier configuration and the reactive permeable barrier. For an operational period of 5 years, the total cost of the bioaugmentation technology would be $816,000 and the reactive barrier would be approximately $1,198,000. After 10 years of operation both technologies would be nearly the same at approximately $1,500,000. If the systems operate 20 years and the barrier material has a life of 10 years, the total cost of the bioaugmentation technology would be $2,871,000 and the reactive barrier would be 2,896,000.

Table 5-6. Present Value Estimates for the Bioaugmentation Technology in the Biobarrier Configuration and Reactive Barrier.

Cost Scenario	Bioaugmentation	Reactive Barrier
Capital Investment Cost	$131,000	$948,000
Annual O&M Cost	137,000	50,000
Present Value over 5 years	816,000	1,198,000
Present Value over 10 years	1,501,000	1,448,000
Present Value over 20 years with 10 year life of barrier	2,871,000	2,896,000

6. Implementation Issues

6.1 Cost Observations

Factors such fouling of the injection well and transfer tubing in both the test and control plots affected the cost of the project at Kelly AFB. Fouling of these system components required additional maintenance costs such as redevelopment of the well and replacement of the tubing. In addition to the costs associated with the repair and replacement of the equipment, the downtime was costly to the project. The fouling of the system components was likely related to the geochemical conditions at the site. Likely oxidation of minerals in the groundwater during the extraction process and precipitation of the mineral in the injection well caused some of the fouling in the wells. Biological growth likely also resulted in some of the fouling of the wells. During full-scale operation, groundwater would not be recirculated, reducing the fouling potential of the wells in the biobarrier. However, for future projects that use a recirculation process, fouling is a potential.

6.2 Performance Observations

The primary objectives of increasing remediation rates compared to biostimulation were achieved with the complete dechlorination that was accomplished with bioaugmentation and the incomplete dechlorination in the control (biostimulation) process. Also, no hazardous materials were produced (accumulated) with the bioaugmentation process; complete dechlorination to ethene occurred. With the complete dechlorination of PCE to ethene, regulatory objectives would have been achieved, and the migration of the contaminant would be minimized. The elimination of the electron donor and the addition of sulfate demonstrated that the added culture were relatively hardy and resistant to perturbation of the aquifer geochemistry.

The objectives of continuous operation were partially achieved. When the system was operating, the groundwater recirculation rates and rates of electron donor addition were relatively constant. However, fouling of the wells caused downtime in the operation.

6.3 Scale-up and Other Significant Observations

Moving the bioaugmentation technology from demonstration-scale to full-scale implementation would require a different application of the technology. As mentioned previously, the full-scale implementation would involve the use of a biobarrier.

The greatest challenges to the successful implementation of a full-scale bioaugmentation project would be the adequate distribution of the microbial culture and the survival of the culture. Proper distribution of the microbial culture is dependent on the physical properties of the aquifer and the application of the culture. In general, more permeable aquifers and greater injection pressures enhance the distribution of the culture. The survival of the culture is primarily dependent on the compatibility of the culture with chemical and biological conditions of the aquifer. The survivability and distribution affect the feasibility of technology more than costs of implementation.

6.4 Lessons Learned

With this demonstration, the bioaugmentation technology was demonstrated to be effective at reducing PCE to ethene at Kelly AFB. While other tests of the bioaugmentation technology have been performed at other locations and with other contaminants, the technology is very site and contaminant specific. The technology also proved unsuccessful at the proposed test site (NAS Fallon). Therefore, additional testing is required for the technology and certainly microcosm testing should be performed at a proposed site prior to conducting full-scale operation.

6.5 End-User Issues

The design and application of bioaugmentation technology consists of installing or using simple components that are readily available. This technology however requires the introduction of organisms specifically selected/grown to operate in subsurface environments where native organisms either are absent or are not robust enough to be simply biostimulated. Initial design and installation of a bioaugmentation system would require some specialized knowledge and it is the express purpose of the Remediation Technologies Development Forum (RTDF) to educate the public with respect to the knowledge needed to appropriately choose such a technology. They can be reached at: http://www.rtdf.org/public/biorem/biodocsp.htm. Several documents have been placed there to assist remedial program managers.

6.6 Approach to Regulatory Compliance and Acceptance

If the technology were implemented in the form of a biobarrier, the regulatory approval to conduct a full-scale bioaugmentation project would likely be limited to underground injection permits (for the culture and the electron donor). Generally, the underground injection permits are authorized by state regulatory agencies. Due to the minimal hazards associated with both the cultures and the electron donating substrates, regulatory approval is likely to be relatively quick.

7. References

United States Environmental Protection Agency, 1996. *Drinking Water Regulations and Health Advisories.* EPA/822-3-96-002. Office of Water, Washington, D.C. October.

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